Advanced Drug Delivery Reviews 229 (2026) 115765

Contents lists available at ScienceDirect

Advanced Drug Delivery Reviews

journal homepage: www.elsevier.com/locate/adr

Engineering tissue patterning in human stem cell-based embryo models☆

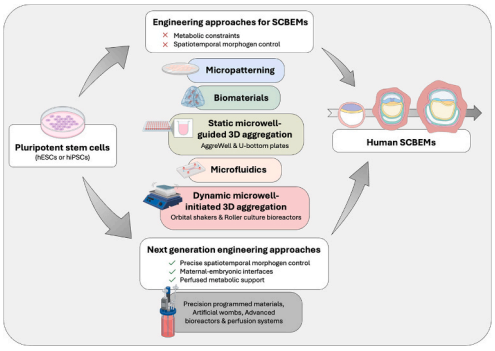
Ella G. Lambert [a], Sara Romanazzo [b,e], Peter L.H. Newman [c,d,*], Kristopher A. Kilian [a,b,d,e,**]

[a] School of Materials Science and Engineering, University of New South Wales (UNSW Sydney), Sydney, NSW 2052, Australia
[b] School of Chemistry, University of New South Wales (UNSW Sydney), Sydney, NSW 2052, Australia
[c] Cancer Ecosystems Program, The Garvan Institute of Medical Research, Darlinghurst, NSW 2010, Australia
[d] School of Clinical Medicine, Faculty of Medicine & Health, University of New South Wales (UNSW Sydney), Sydney, NSW 2052, Australia
[e] Australian Centre for NanoMedicine, University of New South Wales (UNSW Sydney), Sydney, NSW 2052, Australia

HIGHLIGHTS

- Human SCBEMs have revealed previously inaccessible developmental mechanisms.
- Engineering approaches support spatial patterning at native physiological scales in human SCBEMs.
- Standardised engineering-anchored taxonomy enables direct comparison of engineering approaches and biological outcomes.
- Metabolic constraints limit SCBEM complexity and physiological relevance; vascular perfusion systems offer solutions.
- "Moral status by design" framework establishes technical boundaries for responsible SCBEM engineering.

GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
Stem cell-based embryo models (SCBEMs)
Human development
Engineering
Biomaterials
Micropatterning
Microfluidics
Bioreactors

ABSTRACT

Human embryonic development is challenging to study in vitro as animal models inadequately represent human biology, while use of natural human embryos is both ethically and technically limited. Stem cell-based embryo models (SCBEMs) have emerged as a powerful alternative, enabling faithful recapitulation of early human development. However, current approaches predominantly rely on stochastic self-organisation with globally delivered signals, producing variable and often non-recapitulative structures. This review addresses this gap by introducing the first engineering-anchored taxonomy of human SCBEMs, systematically organizing the literature by their underlying technical platform rather than biological outcome alone. We demonstrate how five key engineering approaches – micropatterning, biomaterials, microwells, microfluidics, and dynamic culture – constrain morpho-and-histogenic patterning to determine developmental fidelity. We identify metabolic constraints limiting current models to ~1 mm diameter as the primary bottleneck and demonstrate how vascular engineering and perfusion systems offer solutions. Finally, we propose standardisation metrics linking technical parameters to biological outcomes and establish an ethical framework defined by engineering choices.

☆ This article is part of a Special issue entitled: 'Spatial Pattern in TERM' published in Advanced Drug Delivery Reviews.

* Corresponding author.
** Corresponding author at: School of Materials Science and Engineering, University of New South Wales Sydney, Sydney, NSW 2052, Australia.
E-mail addresses: p.newman@garvan.org.au (P.L.H. Newman), k.kilian@unsw.edu.au (K.A. Kilian).

https://doi.org/10.1016/j.addr.2025.115765
Received 11 August 2025; Received in revised form 16 December 2025; Accepted 19 December 2025
Available online 20 December 2025

1. Introduction

SCBEMs encompass a range of multicellular structures that, through self-organisation and tissue patterning, can partially recapitulate different stages of early embryonic development [1,2]. These models can follow natural developmental programs, mirroring their in vivo counterparts in morphology, transcriptomic profiles, and cellular composition [3,4]. Human SCBEMs have been developed to model a range of developmental stages and events (Figs. 1 and 3, Table 1), including the blastocyst [5–12], early post-implantation embryo [13–21], gastrulation [22–32], amniogenesis [33–36], neurulation [37–44], somitogenesis [45–48], axial formation [49–51], as well as cardiogenesis and neurogenesis [52].

The SCBEM field builds on the studies of human embryogenesis that use scarce natural embryonic material obtained from miscarriages, or donated embryos from in vitro fertilisation procedures that are unable to be used clinically or surplus to patients' needs. Such studies, along with advances for in vitro fertilisation, has led to the establishment of protocols for in vitro culture of human blastocysts up to day 5–6 [53]. However, protocols that extend human embryo culture from peri-to-post-implantation stages remains limited. This is because blastocysts naturally implant into the maternal uterine tissue at around day 7 (Fig. 1) a process that is technically difficult to replicate in vitro. Although researchers have successfully extended in vitro human embryo culture to 12–13 days [54,55], these cultured embryos differ to their in vivo counterparts cellularly and morphologically. Furthermore, extended culture of human embryos is ethically constrained. International guidelines generally prohibit the in vitro growth of human embryos beyond day 14 or the appearance of the primitive streak [2]. Consequently, even in systems capable of culturing post-implantation embryos, the culture period is still limited to comply with the ethical guidelines, restricting the insights that can be gained into post-implantation human development. Given these challenges, significant efforts have been made to develop alternative models, such as SCBEMs, that can provide insights into early human development without relying on natural embryos.

Current approaches to SCBEM generation predominantly use globally delivered exogenous media cues to kickstart the self-organizing feedbacks that drive embryogenesis in stem cells. However, over longer-term cultures, the chaotic dynamics inherent to morphogen feedbacks become increasingly sensitive to small variations in their starting conditions, producing structures that can become increasingly divergent in their phenotypic composition, spatial organisation, and developmental trajectory to their in vivo counterparts.

The inherent stochasticity and lack of precise spatiotemporal control in these current approaches presents an opportunity for engineering approaches to augment SCBEM protocols. In practical terms, successful human SCBEMs emerge when use of engineering approaches help to establish patterned spatial fields: colony size/shape and edge conditions (micropatterns) [31]; diffusive overlays and matrix thickness (biomaterials) [29,34,35,42,56]; initial aggregate diameter/curvature (microwells) [7,9]; or imposed flow and gradient length scales (microfluidics) [25,36,40]. These fields tune patterning at native physiological scales - cell diameters (~10 μm), epithelial sheet and streak widths (100–1000 μm), early axial units such as somites and neural tube diameters (100–1000 μm) with species-specific timing - determining reproducibility and fidelity.

As such, one could use micro-scale patterning (micropatterns, microfluidics) when border placement and axis specification demand cell-to-tens-of-μm control; use mesoscale structuring (microwells, gels) when cavity formation is desired or when curvature or tissue-width programs dominate; or field-level perfusion/dynamics to overcome metabolic ceilings and reveal long-range self-organisation. Hybrid workflows that step from explicit to implicit spatial control (e.g., micropattern to gel embedding to perfusion) most effectively balance resolution, scalability, reproducibility and physiological fidelity for translational SCBEMs.

However, the SCBEM field currently lacks a systematic framework connecting these engineering approaches to biological outcomes, impeding platform selection and reproducibility. This review addresses this gap through three contributions: (1) the first engineering-anchored taxonomy of human SCBEMs, organizing 40+ models by technical platform rather than biological outcome; (2) highlighting relationships between platform-specific spatial control and achievable developmental features; (3) standardisation metrics and ethical frameworks for understanding how engineering choices deliberately constrain or enhance developmental potential.

We provide both a comprehensive reference table (Table 1) and accompanying pictorial representation (Fig. 3) that systematically organises over 40 key human SCBEMs. Table 1 extends on prior reviews in three key ways: (1) it organises human SCBEMs by five core engineering approaches, rather than solely by biological outcome, revealing previously obscured relationships between technical constraints and biological outcomes; (2) it incorporates 22 additional studies to Xue et al. [4], representing the most current and comprehensive synthesis of human SCBEMs available; and (3) it employs emerging standardised nomenclature [57] with engineering prefixes/suffixes that clarify the technical approach used for each SCBEM. While Fig. 3 provides a visual overview of the technological landscape and developmental stages modelled, Table 1 offers detailed methodological specifications including the developmental stage modelled (i.e., SCBEM of… gastrulation, neurulation, amniogenesis etc.), starting cell populations, and key developmental features achieved by the SCBEM. Together, these resources enable researchers to identify optimal technological platforms for specific research questions, compare methodological efficiencies, and recognise gaps in current capabilities. For those entering the field, these complementary tools serve as both a practical protocol selection guide and a strategic overview of how different engineering approaches enable distinct aspects of human development to be recapitulated in vitro.

Throughout the review, we adhere to the standardised nomenclature proposed by Martinez Arias et al. which describe a study by the specific features of embryogenesis that is being modelled – added by either a prefix or suffix (e.g., "SCBEMs of gastrulation") [57]. To aid readers, Fig. 3 showcases a timeline of developmental competence down its left side, which serve the dual purpose of highlighting studies as well as orientating readers to the appropriate terminologies being used for the various SCBEMs we describe throughout. Further, we extend on this nomenclature to include engineering prefixes/suffixes when appropriate. For instance, we classify SCBEMs based on both their developmental stage (blastocyst, gastrulation, somitogenesis, etc) alongside technological approach, i.e. "microfluidic-SCBEM of somitogenesis". Where various studies report their own study specific terminologies, such as "*μNETs (micropatterned neuroepithelial tissues)*", we have adapted the studies language in a manner that we find best preserves the authors intent, while also conforming to the standardised nomenclature. A further discussion on standardisation efforts is provided in Section 3.

Finally, we note that the studies covered below focus on human models alone – this approach has necessarily left many foundational works completed first in mice absent from our analysis. This includes numerous works by pioneering laboratories including those of Rivron [60], Martinez-Arias [61], Wu [62], Zernicka-Goetz [63–70], and Hanna [71] who established key concepts in stem cell self-organisation, morphogenetic control, and embryo model engineering using mouse systems. These foundational mouse studies directly enabled the human SCBEM advances we discuss throughout this review, including innovations in microwell aggregation protocols, dynamic culture systems, and multi-lineage self-organisation that have been successfully translated to human models.

2. Engineering approaches

While coordinating all the distinct local embryological events is

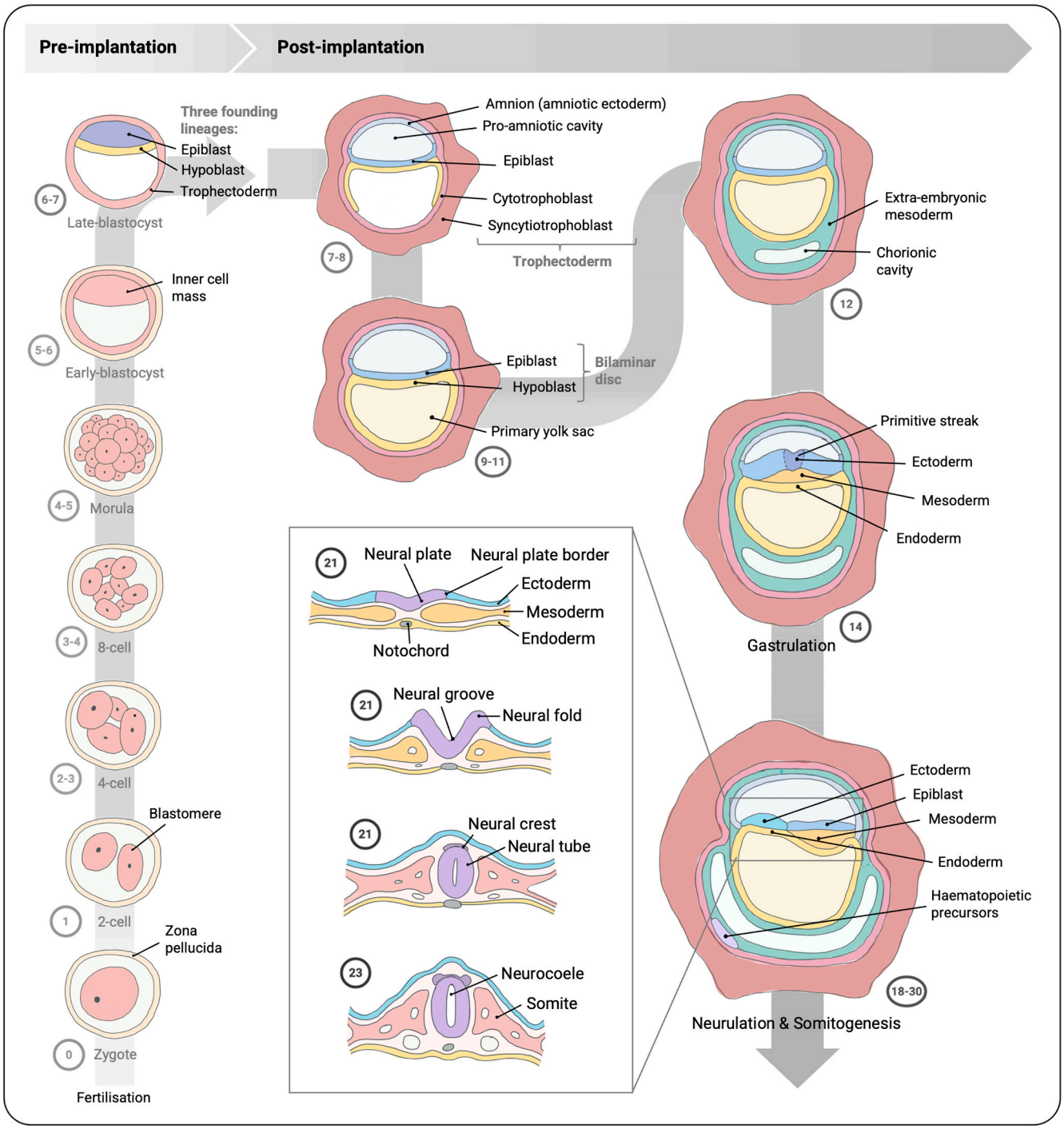


Fig. 1. In vivo, natural human embryo development. Schematic overview of early human embryonic development from pre-implantation to post-implantation development and early morphogenesis. Pre-implantation: Following fertilisation, the zygote undergoes a series of divisions to form 2-, 4-, then 8-cells embryos. At the 16-cell stage, increased cell-cell adhesion leads to morula formation and subsequent cavitation into a blastocyst. Post-implantation: At days 6–7, the three founding lineages emerge: epiblast, hypoblast, and trophectoderm. At around day 7, blastocyst implants into the maternal uterine lining. By days 7–8, the pro-amniotic cavity forms within the epiblast, while the trophoblast differentiates into cytotrophoblast and syncytiotrophoblast. Between days 9–11, the bilaminar disc (epiblast and hypoblast) is established above the primary yolk sac. By day 12, extraembryonic mesoderm and the chorionic cavity begin to develop. At day 14, gastrulation is initiated with the formation of the primitive streak, and epiblast cells ingress to give rise to ectoderm, mesoderm, and endoderm. By days 18–20, the neural plate and notochord form, followed by the emergence of the neural crest (day 21) and the formation of the neurocoele through to full neural tube closure (day 28). Insets show key stages of neurulation and somitogenesis. Circled numbers indicate *days post fertilisation*.

currently out of reach, engineering approaches establish patterned spatial fields, dictating SCBEM formation. Each approach exists on a patterning continuum: from explicit micro-scale patterning (micropatterns; microfluidic gradients) through mesoscale structuring (biomaterial overlays/embeddings; microwell-set aggregate size/curvature) to field-level control (dynamic culture). Each imposes characteristic boundaries or transport regimes that set morphogen, mechanics and cell–cell interaction fields at specific length scales. This section introduces five key engineering approaches: (1) micropatterning, (2) biomaterials and matrix mimics, (3) static microwell-

Table 1
Key SCBEMs organised by engineering approach. Includes categorisation of what the SCBEM is modelling as well as Key developmental features captured, the Starting cell population, and the 'Model name' as described by the authors.

Engineering approach		SCBEM of…	Key developmental features captured	Starting cell population	Model name	Ref
Micropatterning	Laminin-521 on micropatterned glass coverslips	Gastrulation	Trophoblast patterning; Primitive streak patterning; Gastrulation initiation; Triploblastic patterning	Primed hESCs	/	[31,58]
			Trophectoderm specification; Primitive streak patterning; Gastrulation initiation; Triploblastic patterning	Primed hESCs	"Gastruloid"	[23]
	Basement matrix on polyethylene glycol-coated glass coverslips		Trophoblast patterning; Primitive streak patterning; Gastrulation initiation; Triploblastic patterning	Primed hESCs	"Peri-gastrulation-like patterning"	[30]
	Laminin-521 on micropatterned glass coverslips		Primitive streak patterning; Gastrulation initiation; Triploblastic patterning; Mesoderm specification	Primed hESCs	/	[26]
			Trophoblast patterning; Primitive streak patterning; Gastrulation initiation; Triploblastic patterning	Primed hESCs	"Human gastruloid"	[22]
	Basement matrix on PAAm gels on glass coverslips		Primitive streak patterning; Gastrulation initiation; Mesoderm specification	Primed hESCs	"Gastrulation-like node"	[28]
	Vitronectin on PAAm gels on glass coverslips		Primitive streak patterning; Gastrulation initiation; Triploblastic patterning	Primed hiPSCs	/	[29]
	Vitronectin on glass coverslips	Neurulation	Neurectoderm specification; Neural plate border formation; Neural plate formation	hESCs or hiPSCs	"Neurectoderm model"	[42]
	Laminin-521 on micropatterned glass coverslips		Neurectoderm specification; Neural anterior-posterior patterning; Neural dorsal-ventral patterning	Primed hESCs	"Self-organizing neuruloid"	[37]
	Basement matrix on tissue culture plastic		Neurectoderm specification; Neural anterior-posterior patterning;	Primed hESCs or hiPSCs	"μNET" (micropatterned neuroepithelial tissues)	[41]
	Laminin-521 on glass bottom dishes		Neurectoderm specification; Neural plate border formation; Neural plate formation; Neural tube formation; Neural anterior-posterior patterning	Primed hiPSCs	/	[38]
	Basement matrix on glass coverslips	Axial elongation	Neural tube formation; Neural anterior-posterior patterning; Neuromesodermal progenitor specification; Presomitic mesoderm formation; Axial elongation; Somite anterior-posterior patterning; Somite-neural tube coordination	Primed hiPSCs	"Human axial organoid"	[51]
	Basement matrix -coated glass coverslip (>100 μm thickness) + 4 % (*v*/*v*) basement matrix overlay	Amniogenesis	Amniotic cavity formation; Amnion specification	hESCs or hiPSCs	"squamous cysts"	[34]
			Amniotic cavity formation; Bilaminar disc formation; Amnion specification; Amnion-Epiblast boundary formation; Primitive streak formation	hESCs or hiPSCs	"PASE" (post-implantation amniotic sac embryoid)	[35]
	"Gel-3D" (basement matrix bed + basement matrix/ media overlay)	Neurulation	Neurectoderm specification; Neural dorsal-ventral patterning	Primed hESCs or hiPSCs	"NE cyst" (neuroepithelial cyst)	[44]
Biomaterials & matrix mimics	Pyramidal microwells & Basement matrix-coated μ-Slide 8-well (ibidi)	Blastocyst	Trophectoderm specification; Epiblast & hypoblast specification; Blastocyst formation; Trophoblast patterning; Amniotic cavity formation (extended culture); Yolk sac cavity formation (extended culture)	Naïve hESCs or hiPSCs	"Blastoid"	[11]
	Pyramidal microwells & Basement matrix (1:1 Geltrex™/Matrigel™) (extended blastoid culture)		Trophectoderm specification; Blastocyst formation; Epiblast & hypoblast specification; Trophoblast patterning; Amniotic cavity formation; Yolk sac cavity formation; Bilaminar disc formation; Primordial germ cell specification; Primitive streak patterning;	Naïve hESCs	"3D-cultured blastoid"	[8]

(*continued on next page*)

Table 1 (*continued*)

Engineering approach		SCBEM of…	Key developmental features captured	Starting cell population	Model name	Ref
			Gastrulation initiation; Extraembryonic mesoderm specification			
	Basement matrix/poly(N-isopropylacrylamide) (1:5) Or Basement matrix/Biogelx (1:5)		Epiblast anterior-posterior patterning; Primitive streak patterning; Gastrulation initiation	hESCs	"3D human epiblast model"	[18]
	Assembloid approach: 3D epiblast cysts in basement matrix/poly(N-isopropylacrylamide) (1:5) Extraembryonic-like cells on 'soft-substrates' (laminin-521-coated PDMS (1.5 kPa))	Post-implantation embryo	Epiblast anterior-posterior patterning; Trophoblast patterning; Primitive streak patterning; Gastrulation initiation; Extraembryonic mesoderm specification	Primed hESC-derived 3D epiblast cysts + Primed BMP4-hESCs (xEM-like cells)	"Human embryoids" / "embryo assembloids"	[19]
	U-bottom low-adherence well plate +10 % basement matrix (embedded culture)		Neuromesodermal progenitor specification; Presomitic mesoderm formation; Axial elongation; Somite anterior-posterior patterning; Somite epithelisation	Primed hiPSCs	"Somitoid"	[47]
	10 % growth factor-reduced basement matrix		Neuromesodermal progenitor specification; Presomitic mesoderm formation; Axial elongation; Somite anterior-posterior patterning; Somite epithelisation	hiPSCs	"Axioloid"	[48]
	96-well U-bottom plate + laminin-521 or gelatin coated 2D surfaces (ibidi polymer or glass)	Somitogenesis	Presomitic mesoderm formation; Axial elongation; Somite anterior-posterior patterning; Somite epithelisation	hiPSCs	"Somitoid"	[46]
	96-well cell-repellent U-bottom plate +10 % basement matrix (embedded culture)		Neuromesodermal progenitor specification; Presomitic mesoderm formation; Axial elongation; Somite anterior-posterior patterning; Somite epithelisation	hiPSCs	"Segmentoid"	[46]
	96-well plate +5 % basement matrix (embedded culture)		Triploblastic patterning; Neurectoderm specification; Neural anterior-posterior patterning; Neural dorsal-ventral patterning; Axial mesoderm anterior-posterior patterning; Neuromesodermal progenitor specification; Presomitic mesoderm formation; Axial elongation; Somite epithelisation; Somite-neural tube coordination	Primed hESCs or hiPSCs	"RA-gastruloid"	[50]
	50 % growth factor-reduced basement matrix (embedded culture)	Axial elongation	Neuromesodermal progenitor specification; Axial elongation; Somite anterior-posterior patterning; Somite epithelisation; Somite-neural tube coordination	Primed hiPSCs	"Trunk organoid"	[49]
			Trophectoderm specification; Epiblast & hypoblast specification; Blastocyst formation; Amniotic cavity formation (extended culture); Yolk sac cavity formation (extended culture)	Naïve hESCs or hiPSCs	"Blastoid"	[12]
Static microwell-guided 3D aggregation	Pyramidal microwells	Blastocyst	Trophectoderm specification; Epiblast & hypoblast specification; Blastocyst formation; Amniotic cavity formation (extended culture)	hiPSC intermediates (partially reprogrammed from fibroblasts)	"iBlastoid"	[9]
			Trophectoderm specification; Epiblast & hypoblast specification; Blastocyst formation;	hEPSCs (untreated) + BMP4- hEPSCs (Trophectoderm-like cells)	"EPS-blastoid"	[5]
			Trophectoderm specification; Blastocyst formation; Epiblast & hypoblast specification;	hEPSCs	"Blastoid"	[10]
	Microwell array (in 96-well plate)		Trophectoderm specification; Blastocyst formation; Epiblast &	Naïve hESCs (Hippo, TGF-β2, ERK inhibited)	"Blastoid"	[7]

(*continued on next page*)

Table 1 (*continued*)

Engineering approach		SCBEM of…	Key developmental features captured	Starting cell population	Model name	Ref
			hypoblast specification; Amniotic cavity formation (extended culture); Amnion specification (extended culture)			
	Pyramidal microwells or Suspension plates + basement matrix-coated 8-well slides (extended culture)		Trophectoderm specification; Blastocyst formation; Epiblast & hypoblast specification; Primitive streak patterning (attached culture)	Naïve hESCs	"spontaneous blastoids"	[6]
			Epiblast & hypoblast specification; Amniotic cavity formation; Yolk sac cavity formation; Bilaminar disc formation; Extraembryonic mesoderm specification	hESCs + SNCs (BMP4/SB431542 induced hESCs)	"*E*-assembloid"	[13]
	Pyramidal microwells	Post-implantation embryo	Trophectoderm specification; Epiblast & hypoblast specification; Amnion specification; Primordial germ cell specification; Extraembryonic mesoderm specification	Naïve hESCs + iGATA6-SOX17 hESC + iGATA3-AP2γ hESC	"hiEmbryoids"	[20,59]
	Pyramidal microwells + basement matrix embedding (extended culture)		Epiblast & hypoblast specification; Epiblast anterior-posterior patterning; Yolk sac cavity formation; Primitive streak patterning	Intermediate hESCs	"Extra-embryoids"	[17]
	Microwell array (in 12-well or 96-well plate)		Amniotic cavity formation; Epiblast anterior-posterior patterning; Amnion specification; Primordial germ cell specification; Primitive streak patterning	Naïve hESCs or naïve hiPSCs + nHyCs	"Bilaminoids"	[15]
	Pyramidal microwells		Epiblast anterior-posterior patterning; Amniotic cavity formation; Yolk sac cavity formation; Bilaminar disc formation; Extraembryonic mesoderm specification; Haematopoiesis initiation	Primed hiPSCs +iGATA6 hiPSCs	"heX-embryoid"	[14]
	U-bottom low-adherence 96-well plate	Amniogenesis	Amniotic cavity formation; Yolk sac cavity formation; Extraembryonic mesoderm specification	Primed hiPSCs or hESCs	"PGA" (post-gastrulation amnioid)	[33]
			Triploblastic patterning; Axial elongation	Primed hESCs	"Gastruloid" (3D)	[27]
			Amniotic cavity formation; Yolk sac cavity formation; Bilaminar disc formation; Primordial germ cell specification; Primitive streak formation; Extraembryonic mesoderm specification; Haematopoiesis initiation	Primed hiPSCs or hESCs	"Gastruloid" (3D)	[32]
	Pyramidal microwells	Gastrulation	Amniotic cavity formation; Yolk sac cavity formation; Bilaminar disc formation; Epiblast anterior-posterior patterning; Primordial germ cell specification; Primitive streak formation; Gastrulation initiation; Trilaminar disc formation; Extraembryonic mesoderm specification; Haematopoiesis initiation; Neuroectoderm specification; Neural tube formation; Neural anterior-posterior patterning	hEPSCs	"Peri-gastruloid"	[24]
Microfluidics	Microfluidics (BMP4 & NOGGIN gradients)	Gastrulation	Trophoblast patterning; Epiblast anterior-posterior patterning; Primitive streak patterning; Gastrulation initiation; Extraembryonic mesoderm specification; Triploblastic patterning;	Primed hESCs or hiPSCs	/	[25]
	Microfluidics (basement matrix pockets)	Amniogenesis	Amniotic cavity formation; Amnion specification; Amnion-epiblast boundary formation; Epiblast anterior-posterior patterning; Primordial germ cell	Primed hESCs or hiPSCs	"microfluidic human embryoid"	[36]

(*continued on next page*)

Table 1 (*continued*)

Engineering approach		SCBEM of…	Key developmental features captured	Starting cell population	Model name	Ref
			specification; Primitive streak patterning; Gastrulation initiation			
	Microfluidics (WNT gradient)		Neural plate formation; Neural anterior-posterior patterning; Neural dorsal-ventral patterning	hESCs and hiPSCs	"MiSTR tissue" (microfluidic-controlled stem cell regionalization)	[40]
	Basement matrix microcontact printing + Microfluidics (retinoic acid, FGF2, WNT gradients)	Neurulation	Neuroectoderm specification; Neural plate border formation; Neural tube formation; Neural anterior-posterior patterning; Neural dorsal-ventral patterning; Neuromesodermal progenitor specification; Spinal cord dorsal-ventral patterning;	Primed hESCs or hiPSCs	"μNTLS" (microfluidic NT-like structure)	[43]
	Microfluidics + basement matrix embedding (retinoic acid, FGF2, WNT gradients)	Somitogenesis	Presomitic mesoderm formation; Axial elongation; Somite anterior-posterior patterning; Somite epithelisation	PSMs (hESCs or hiPSC derived)	"μSDM" (microfluidic somite development model)	[45]
	6-well plate (with anti-adherence coating) on Orbital shaker	Neurulation	Endoderm-mesoderm boundary formation; Primitive gut tube formation; Neuromesodermal progenitor specification; Axial elongation; Spinal cord dorsal-ventral patterning; Neural crest migration	Primed hiPSCs	"EMLO gastruloid" (elongating multi-lineage organised)	[39]
		Cardiogenesis & Neurogenesis	Neuroectoderm specification; Cardiac crescent formation; Heart tube formation; Primitive gut tube formation; Neural anterior-posterior patterning; Axial elongation; Neural crest migration	Primed hiPSCs	"EMLOC gastruloids" (elongating multi-lineage organised cardiac)	[52]
Dynamic microwell-initiated 3D aggregation	Pyramidal microwells +6-well plate on Orbital shaker (+ roller culture (optional))		Trophectoderm specification; Epiblast & hypoblast specification; Trophoblast specification; Amniotic cavity formation; Yolk sac cavity formation; Bilaminar disc formation; Amnion specification; Chorionic cavity formation; Connecting stalk formation; Epiblast anterior-posterior patterning; Primordial germ cell specification; Extraembryonic mesoderm specification	Naïve hESCs + PE/ExM-like cells (RCL media induced) + TE-like cells (BAP(J) induced)	"SEM""(structured stem-cell-based embryo model)	[16]
	Pyramidal microwells +6-well suspension culture plate (with anti-adherence coating) on Tissue shaker	Post-implantation embryo	Trophectoderm specification; Epiblast & hypoblast specification; Trophoblast patterning; Amniotic cavity formation; Yolk sac formation; Bilaminar disc formation; Amnion specification; Chorionic cavity formation; Connecting stalk formation; Epiblast anterior-posterior patterning; Primordial germ cell specification; Primitive streak formation; Gastrulation initiation; Extraembryonic mesoderm specification; Mesoderm specification	SACs (STAT3-activated cells) (hESC or hiPSC derived)	"stEMs" (STAT3-mediated embryo model)	[21]

guided 3D aggregation, (4) microfluidics, and (5) dynamic microwell-initiated 3D aggregation - and discusses the SCBEMs they generate (Fig. 3, Table 1).

2.1. Micropatterning

Micropatterning spatially confines cells through the patterning of proteins or extracellular matrices in defined geometric features, typically on 2D glass or hydrogel surfaces (Fig. 2). This approach provides precise control over cell colony size and geometry, enabling reproducible differentiation patterning that is difficult to achieve in standard 2D cultures. With 10s-μm precision, micropatterns can set geometric and edge-sensing cues at scales relevant to early epithelial sheet patterning and primitive-streak-scale events (protein features ~50–1000 μm; reproducible ring widths ~100–1000 μm) [31,58]. This enables deterministic radial or axial organisation with high well-to-well reproducibility and plate-level scalability. The addition of thin gel overlays can extend pattern fidelity into 3D (lumen/curvature) while retaining imposed boundaries [38]. Many SCBEMs, including the early landmark SCBEMs of gastrulation [22,23,26,28–31], as well as more recent SCBEMs of neurulation [37,38,41,42] and axial elongation [51], were generated through the micropatterning of human induced pluripotent stem cells (hiPSCs) and human embryonic stem cells (hESCs), and have helped uncover various signalling, self-organisation and developmental patterning processes. Though planar bias and diffusion-limited delivery (unless combined with flows) are notable limitations of micropatterning, the reproducibility and precise spatial control make it the most reliable platform for investigating signal–mechanics interactions at native tissue widths.

Many of the first SCBEMs were micropatterned-SCBEMs of gastrulation. These early SCBEMs relied on the micropatterning of hESCs on laminin-521 coated, commercially available, circular micropatterned substrates [31,58]. When confined and treated with *bone morphogenetic protein* (BMP) *4*, the hESCs differentiated and self-organised into concentric triploblastic (three germ layers), radial patterns, with an ectoderm-like centre, primitive/mesoderm-like ring, endoderm-like ring, and trophectoderm-like ring at the edges. This reproducible differentiation and spatial organisation was a landmark achievement for SCBEMs, showing how geometry and confinement could promote tissue patterning with globally administered morphogen fields.

Building on this came a range of micropatterned-SCBEMs of gastrulation, all using laminin-521 micropatterned glass. These SCBEMs featured triploblastic patterning, primitive streak patterning, and trophectoderm or trophoblast patterning, and helped elucidate various developmental mechanisms [22,23,26]. The micropatterned-SCBEMs by Etoc et al. demonstrated that cell density and position within colonies systematically controls *transforming growth factor-beta 2* (TGF-β2) receptor localisation and signalling response, wherein at high densities, TGF-β2 receptors re-localise from apical to lateral cell surfaces, making cells insensitive to apically-applied signals [23]. Martyn et al. demonstrated the crucial roles of *wingless-related integration* (WNT) *site 3* and NODAL signalling protein in inducing primitive streak and organiser formation within their micropatterned-SCBEMs [26]. Instead of BMP4 treatment, they showed that WNT3 alone could induce radial patterning of hESCs, with a pluripotent epiblast-like centre and primitive streak-like edge, while combined WNT3 and Activin A treatment also induced an organiser region at the edge. This study provided compelling evidence of functional organiser activities, and self-organizing embryonic structures, thus advancing the functional understanding of human gastrulation. Chhabra et al. investigated the signalling dynamics in confined hESCs more closely, by quantifying the temporal and spatial interplay among BMP, WNT, and NODAL signalling pathways [22]. They showed that BMP initiated wave-like dynamics in WNT and NODAL activities, suggesting a dynamic rather than static signalling landscape, challenging previous gradient-based interpretations. They concluded that mesoderm differentiation relied on the temporal dynamics of signalling rather than spatial gradients alone.

Tewary et al. generated micropatterned-SCBEMs of gastrulation by using ultraviolet (UV) photolithography to micropattern basement matrix (i.e., Geltrex™, Matrigel™, Cultrex™) on polyethylene glycol-coated glass coverslips [30]. They showed that micropatterned hESCs responded to BMP4 in a dose-dependent manner, and that the radial patterning followed a combination of reaction-diffusion mechanisms and positional information.

Studies of primitive streak formation that better recapitulate embryonic mechanobiology have been motivated by observations that softening of the basement membrane precedes primitive streak formation alongside triploblastic specification [72,73]. This has inspired investigations into how substrate mechanics influence pluripotent stem cell fate decisions.

Previous work demonstrated that materials-based mechanical perturbation can modulate cellular responses to morphogenetic signals [74–77]. Muncie et al. generated micropatterned-SCBEMs of gastrulation, elegantly showing that geometric confinement and mechanical tension spatially pattern BMP4-induced mesoderm specification, with tension-mediated β-catenin release sensitising cells to exogenous differentiation factors [28]. Newman et al. expanded on this work, generating 3D printed-SCBEMs of the primitive streak and germ layer patterning, through culture of hiPSCs on structured stiffness gradients (Fig. 4E) [56]. Srivastava et al. used micropatterning on polyacrylamide hydrogels of varying stiffness ($1 < E < 100$ kPa) to generate SCBEMs of primitive streak formation and germ layer patterning. They found that the stress at the boundary induces a contractile phenotype, with enhanced proliferation and appearance of a primitive streak-like

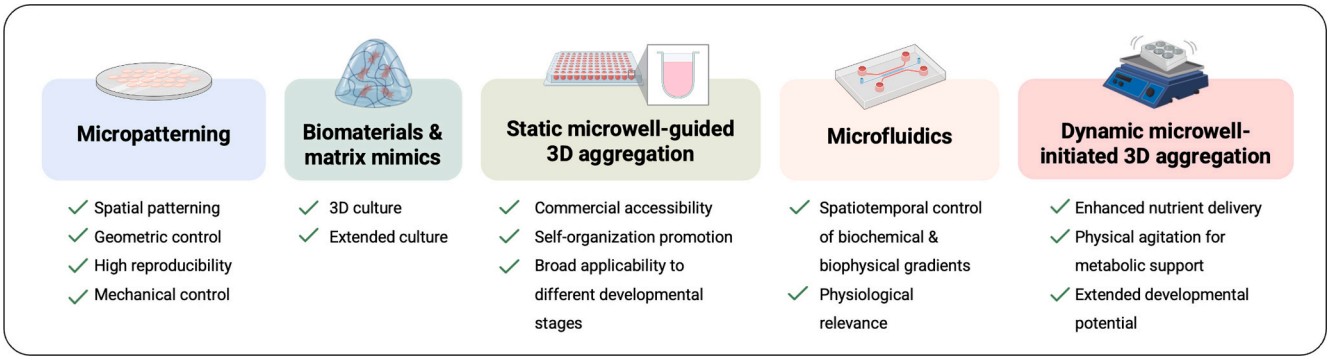


Fig. 2. Advantages of key engineering approaches for SCBEMs. Schematic illustrating five major engineering approaches and their advantages for generating SCBEMs: micropatterning, biomaterials and matrix mimics, static microwell-guided 3D aggregation, microfluidics, and dynamic microwell-initiated 3D aggregation. These approaches vary in their ability to control spatial organisation, mechanical cues, and biochemical gradients, leading to differences in their recapitulation of developmental patterning and morphogenesis in vitro, and thus in the SCBEMs they can generate.

mesendodermal population, without the addition of soluble BMP4 [29]. Confinement on a deformable matrix promoted cytoplasmic translocation of yes-associated protein 1 with endogenous non-canonical WNT signalling coordinating multilayered differentiation within 48 h (Fig. 4A). This demonstrated how the properties of the cell culture substrate can greatly influence cell behaviour and provides scope for materials design to replicate morphogenetic processes, initiating endogenous signalling gradients to drive differentiation. These collective findings highlight the bidirectional interplay between the mechanical microenvironment and the soluble signals that together direct morphogenesis.

Following the success of micropatterned-SCBEMs of gastrulation, several groups adapted this approach to recapitulate human neurulation events [37,38,41,42]. Xue et al. [42] generated a micropatterned-SCBEM of neuroectoderm specification in circular colonies with neural induction media. They demonstrated how cell shape and cytoskeletal contractility guide neuroectoderm patterning through BMP and *suppressor of mother against regulated decapentaplegic* (SMAD) signalling, achieving a model with neuroepithelial and neural plate border patterning. Haremaki et al. [37] used a similar approach to generate micropatterned-SCBEMs of the neural tube. However, the addition of a dual-SMAD inhibition with BMP4 stimulation enabled the generation of highly reproducible structures containing various ectodermal derivatives (neural progenitors, neural crest, sensory placodes, and epidermis) in concentric patterns, which they successfully applied to model Huntington's disease.

Advancing beyond 2D patterning, Karzbrun et al. [38] developed micropatterns followed by use of basement matrix to create a 3D microenvironment, to generate micropatterned-SCBEMs of the neural tube. They first micropatterned hiPSCs in rectangular patterns, before adding basement matrix which triggered the initially 2D hiPSC sheet to transition to millimetre- long 3D tissue surrounding a lumen. Following neural induction, this 3D tissue surrounding a lumen underwent neural patterning and folding to generate a neural tube-like structure surrounded by surface ectoderm. Similarly, Sahni et al. [41] created micropatterned-SCBEMs of neuroectoderm specification using mesoendoderm priming followed by neural induction. These micropatterned-SCBEMs exhibited invagination-like folding and *E*-to-N-cadherin switching, with TGF-β2 signalling from adjacent endodermal cells supporting tissue morphogenesis.

Finally, most recently Yaman et al. generated micropatterned-SCBEMs of axial elongation, modelling the trunk region of an embryo containing the spinal cord and somites, characterised by the co-development of the neural tube with somites [51,57]. They micropatterned hiPSCs in 150 μm groups of four at vertices of 200 × 200 μm squares, a design optimised by machine learning [78] for desired organoid morphology, specifically for anterior-posterior symmetry breaking with proper arrangement of neuromesodermal progenitors, paraxial mesoderm, and neural tube cells. The micropatterned colonies were then exposed to differentiation media that included basement matrix supplementation (6 % *v*/v) and later transferral to low-attachment well plates for further development in a permissive 3D environment. This approach enabled Yaman et al. to generate hundreds of axially elongated SCBEMs at once that reliably developed neural tube-like structures flanked by segmented somites.

2.2. Biomaterials & matrix mimics

Biomaterials and matrix mimics can play a crucial role in SCBEM development by providing a 3D microenvironment to support proper cellular self-organisation and differentiation (Fig. 2). The choice of biomaterial directly influences cell fate decisions, morphogenesis, and the fidelity of developmental processes recapitulated in vitro. Despite the importance of the extracellular matrix, current SCBEMs have relied on a surprisingly narrow range of commercially available, natural extracellular matrix-derived hydrogels, namely laminin-521 [46] and basement membrane-derived matrices (i.e., Matrigel™, Geltrex™, and Cultrex™) which are henceforth referred to as basement matrix [8,11,18,19,34,35,44,47–50]. These materials, while limited in chemical definition and batch variability, have proven remarkably effective at supporting complex developmental processes.

Several groups have generated SCBEMs using biomaterials in a "sandwich" culture system whereby pluripotent cells are seeded onto a 2D basement matrix surface, then later overlaid with basement matrix. Shao et al. used this approach to generate two distinct SCBEMs of amniogenesis [34,35]. Their method involved seeding hESCs on a 2D basement matrix bed, followed by a 3D overlay of media containing 4 % (*v*/v) basement matrix after 24 h. This simple biomaterial-based strategy was sufficient to induce self-organisation into SCBEMs featuring amniotic cavities and further amnion specification. Remarkably, their later SCBEM [35] also exhibited bilaminar disc formation and primitive streak patterning, without exogenous morphogens or maternal tissues.

Zheng et al. adapted this 'sandwich' approach for neural development, generating a SCBEM of the neural tube [44]. By combining the basement matrix 'sandwich' system with neural induction media, they established a 'neurogenic environment' and generated dorsal-ventral patterned, spherical, luminal cysts that mirrored parts of the neural plate in the dorsal ectoderm.

While biomaterials alone rarely support complete SCBEM generation, combining initial 3D aggregation in systems such as pyramidal microwells (Section 2.3), followed by extended biomaterials-supported culture, has proven highly effective. This two-step approach allows for controlled initial cell organisation and aggregation, followed by biomaterial-mediated morphogenesis.

For example, Yanagida et al. [11] generated SCBEMs of the blastocyst by first aggregating naïve hESCs or hiPSCs in pyramidal microwells to form SCBEMs of the blastocyst complete with epiblast, hypoblast and trophectoderm segregation, followed by their transfer onto basement matrix-coated dishes on day 4, for an additional 4 days of 'extended attached' culture. This extended culture supported the outgrowth of trophoblast-like cells, as well as the formation of yolk sac-like and amnion-like cavities. Similarly, Karvas et al. generated SCBEMs of the blastocyst by aggregation of naïve hESCs in pyramidal microwells to generate blastocyst-like structures, followed by extended culture of these structures on 1-2 mm thick sheets of basement matrix [8] (Fig. 4C). This approach supported the SCBEMs' continuous development across 21 days, from pre-implantation through to early gastrulation stages, producing amniotic-like- and yolk sac-like cavities, bilaminar disc formation, primitive streak patterning, gastrulation initiation, as well as primordial germ cell and extraembryonic mesoderm specification. They also showed that the ratio of basement matrix product can bias lineage outcomes, with Matrigel™ matrices promoting higher expression of trophoblast markers in SCBEMs compared to Geltrex™, whereas the 1:1 Matrigel™: Geltrex™ combination supported a more proportionate development of epiblast, hypoblast, and trophectoderm lineages.

The combinatorial approach has also been particularly successful for modelling later or more specific developmental stages, with multiple groups have generating SCBEMs of somitogenesis [46–48], and SCBEMs of axial formation [49,50] through aggregation followed by basement matrix supplementation or embedding.

Sanaki-Matsumiya et al. developed a SCBEM of somitogenesis by aggregating hiPSCs in U-bottom plates with 10 % basement matrix added on day 4 [47]. These SCBEMs demonstrated periodic formation of epithelised somite structures with rostral and caudal compartments, proper presomitic mesoderm and neuromesodermal progenitor arrangement along the anterior-posterior axis, and segmentation clock oscillations. They demonstrated that while somite cell differentiation and axial elongation could occur in the absence of basement matrix, basement matrix was critical for epithelised somite formation with proper apical-basal polarity. Similarly, Miao et al. developed two different SCBEMs of somitogenesis using biomaterials [46]. Both these somitogenesis SCBEMs were generated by first aggregating hiPSCs in

low-attachment U-bottom wells, however on day 2, the SCBEMs were either transferred onto laminin-521- or gelatin-coated dishes to promote polarised somite rosette formation, or embedded in in 10 % basement matrix droplets to promote axial elongation and further neuro-mesodermal patterning. Yamanaka et al. generated mesoderm-based SCBEMs of axial elongation with biomaterials [48]. These SCBEMs were generated by priming hiPSCs toward a mesoderm fate, followed by 3D aggregation where the aggregates broke symmetry and underwent axial elongation before being embedded in 10 % growth factor-reduced basement matrix at 72 h. Interestingly, unlike Sanaki-Matsumiya et al. [47], Yamanaka et al. showed that while basement matrix alone supported axial elongation and initial segment formation, it was insufficient for generating properly epithelised somite structures. Only with the addition of retinoic acid treatment alongside basement matrix, were they able to stabilise the segments and demonstrate development of epithelised somites with clear apical-basal polarity.

Building on this came two SCBEMs of axial formation formed by first using 3D static aggregation, followed by embedded basement matrix culture [49,50]. Hamazaki et al. generated SCBEMs of gastrulation and axial elongation through 3D aggregation of hESCs or hiPSCs with early retinoic acid exposure (0–24 h) followed by 5 % basement matrix supplementation at 48 h [50]. The resulting SCBEMs featured neural tubes flanked by segmented somites and contained diverse cell types from all three germ layers, including neural crest cells, neural progenitors, renal progenitors, and myocytes. Gribaudo et al. used a similar approach with 3D aggregation of hiPSCs in pyramidal microwells, followed by embedded culture 50 % growth factor-reduced basement matrix to generate SCBEMs of axial elongation [49]. These SCBEMs showed a patterned neural tube flanked by segmented somites, as well as further maturation to include terminal cell types including neuronal projections contacting spine and muscle progenitors.

Finally, unlike prior biomaterial approaches for SCBEMs, which were largely limited to laminin-521 and basement matrix extracts, Simunovic et al. used a combination of basement matrix and engineered polymers to generate a SCBEM of the post-implantation epiblast [18], and a SCBEM of the gastrulating post-implantation embryo [19]. The biomaterial-based epiblast SCBEM was made by embedding hESCs in a composite hydrogel of basement matrix with a temperature-responsive synthetic hydrogel (polyethylene glycol-based or 9-fluorenylmethyloxy-carbonyl-based) in a 1:5 ratio [18]. At 4 °C the hydrogel mixture remained liquid so the cells could easily be embedded, and upon warming to 37 °C, the mixture solidified into a soft gel, supporting the formation of polarised epiblast cysts. Combined with BMP4 treatment, this approach supported symmetry breaking and primitive streak-like patterning in the SCBEM. Further leveraging the temperature-responsive properties of the hydrogel, Simunovic et al. were able to retrieve the epiblast cysts, and assemble them with extraembryonic-like cells to generate biomaterial-SCBEMs of gastrulation initiation [19]. In these models, the epiblast cyst became enveloped by the extraembryonic layer and, upon attachment to tissue culture substrates, broke symmetry and initiated gastrulation-like processes, exhibiting spatial expression, all without further exogenous morphogens. This two-step gel embedding and recovery strategy thus provides both a way to form and maintain a pluripotent, polarised epiblast spheroid and integration with extraembryonic cells to model post-attachment human embryogenesis in vitro.

2.3. Static microwell-guided 3D aggregation

Static microwell-guided 3D aggregation is a widely used approach for generating human SCBEMs (Fig. 2, Table 1), demonstrating that complex developmental programs can emerge from relatively simple initial conditions (Fig. 3). This approach uses broadly available tissue culture microwell platforms, such as pyramidal microwell plates and non-adherent U-bottom well plates, to force 3D cell aggregation and promote self-organisation. In this approach, spatial programming is implicit: microwells impose starting conditions – aggregate diameter (150–800 μm), cell–cell ratios, and curvature radius – that seed endogenous morphogen feedbacks at tissue scales. This geometry-driven control, combined with global biochemical cues and endogenous gradients, enables tissue-scale uniformity with very high throughput (96–384-well format), making the method excellent for multi-day stage progression studies and screening applications. Despite widespread adoption of microwells for generating SCBEMs of blastocyst through early organogenesis [5–7,9,10,12–15,17,20,24,27,32,33], this approach faces persistent challenges of low efficiency and high structural variability [3]. Additionally, deterministic localization of specific borders requires hybridisation with approaches such as micropatterning, biomaterials, or microfluidic gradient devices. In this section we discuss the key human SCBEMs that have used static microwell-guided 3D aggregation.

The microwell aggregation approaches used for human SCBEMs build directly on foundational mouse studies by Rivron [60], Zernicka-Goetz [63,64], and others that established the principles of controlled stem cell self-organisation into blastocyst-like structures. Many prominent SCBEMs of the blastocyst have been generated using static microwell aggregation, though protocols differ, such as in their media formulations, starting cell populations and size aggregate size.

Yu et al. generated SCBEMs of the blastocyst [12]. These SCBEMs were produced by sequentially treating naïve hESCs or hiPSCs with hypoblast differentiation medium followed by trophoblast differentiation medium, or vice versa, in pyramidal microwells. By days 6–8 post aggregation (depending on the media sequence), the SCBEMs showed segregation of the epiblast, hypoblast and trophectoderm-like cells, and resembled an embryonic day 6 (E6) natural embryos, in size and composition. Extending for an additional 4 days led to the development of amniotic-like and yolk sac-like cavities in some SCBEMs, resembling day 9–10 natural embryos.

Liu et al. generated an induced SCBEM of the blastocyst termed *"iBlastoids"* [9]. Rather than using commercially available hESC or hiPSC lines, they used day 21 Sendai-virus (*octamer-binding transcription factor 4* (Oct4), *kruppel Like Factor 4* (Klf4), *Sry-box 2* (Sox2), and *cellular myelocytomatosis oncogene* (c-MYC)) reprogrammed intermediates (not fully reprogrammed iPSCs). These cells were aggregated in pyramidal microwells and as early as day 6 post aggregation, SCBEMs emerged showing blastocyst-like cavitation and segregation of the epiblast, hypoblast and putative trophectoderm-like cells, comparable to day 5–7 natural embryos in size and shape. However, subsequent comprehensive transcriptomic reanalysis using improved reference datasets has challenged the initial cellular characterisation claims. Zhao et al. demonstrated that cells initially identified as trophectoderm-like in Liu et al.'s SCBEMs actually express amnion markers (*ISL LIM Homeobox 1* (ISL1), *Gamma-Aminobutyric Acid Type A Receptor Pi Subunit* (GABRP), *Insulin-Like Growth Factor Binding Protein 7* (IGFBP7)) rather than trophectoderm markers (*GATA Binding Protein 2* (GATA2), *Glial Cells Missing Homolog 1*(GCM1), *Bridging Integrator 2* (BIN2)), and cluster transcriptionally with amnion rather than trophectoderm lineages when compared against comprehensive human embryo references [79]. Attachment assays demonstrated that Liu et al.'s SCBEMs of blastocyst could model early implantation stage in vitro, with epiblast-like cell expansion, pro-amniotic-like cavity formation, trophectoderm-like cell differentiation into trophoblast-like cell states (syncytiotrophoblast-like and extravillous cytotrophoblast-like cells), and primitive endoderm-like cells at the perimeter of the epiblast-like compartments. While these models retain value for studying certain aspects of early development, the cellular composition differs from initial reports, highlighting the importance of rigorous transcriptomic validation against comprehensive embryonic references for proper characterisation of embryo models.

Later that year, Fan et al. developed static microwell-SCBEM of the blastocyst using a two-step protocol where hiPSC derived human extended (or expanded) pluripotent stem cells (hEPSCs) were pre-treated with BMP4 to generate trophectoderm-like cells, then mixed

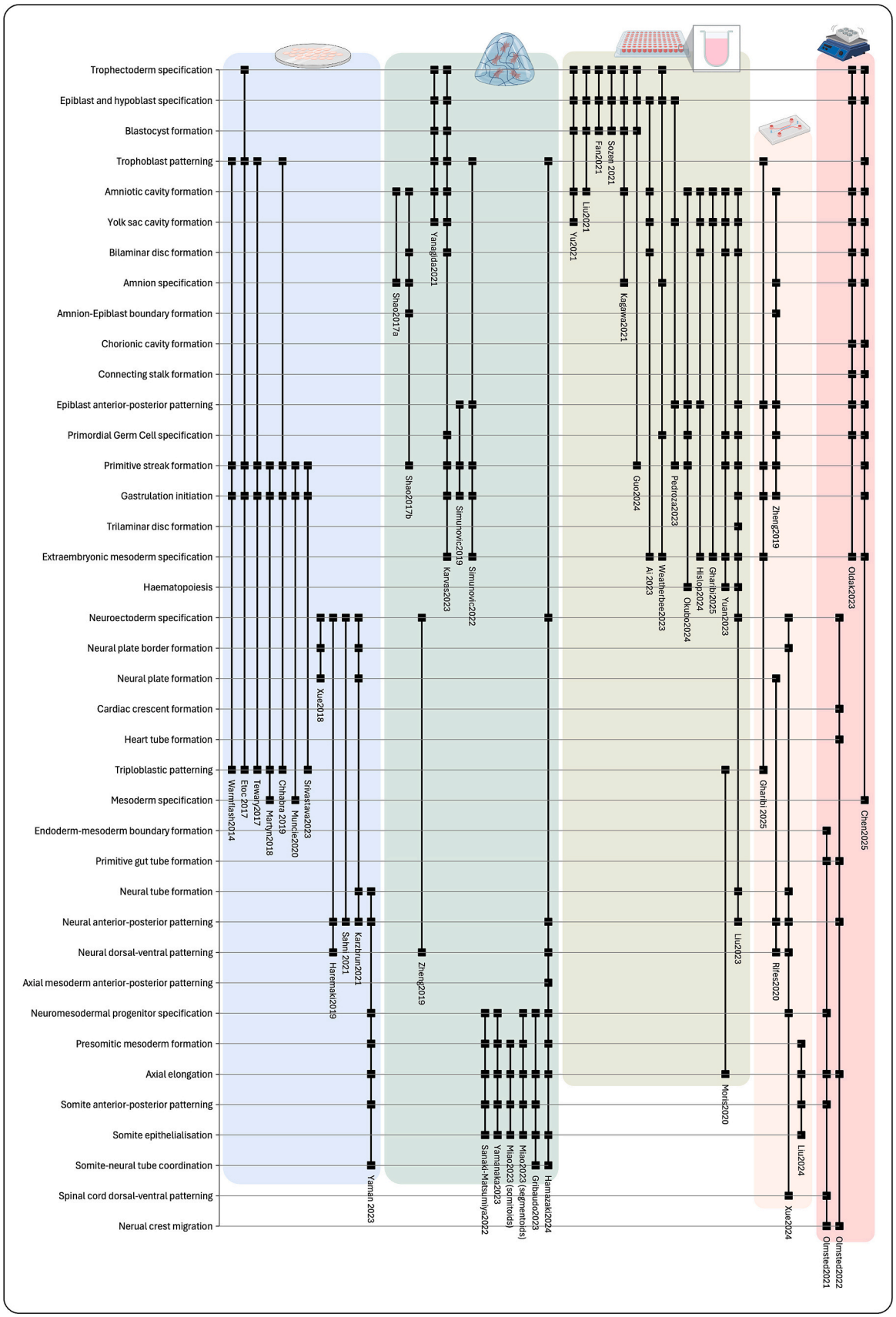


Fig. 3. Key human SCBEMs, organised by Engineering approach and developmental events modelled. Colour = Engineering approach (blue = micropatterning, green = biomaterials & matrix mimics, khaki = static microwell-guided 3D aggregation, peach = microfluidics, red = dynamic microwell-initiated 3D aggregation.

with untreated hEPSCs (1:4–1:5 ratio) before aggregation [5]. This approach produced blastocyst-like structures with lineage segregation of the epiblast, hypoblast, and trophectoderm by day 5–6. Extended culture with basement matrix for up to 8–10 days, revealed transcriptomic similarities to natural embryos cultured in vitro for 7–14 days post fertilisation. Sozen et al. also generated a static microwell-SCBEM of the blastocyst using hESPCs, albeit a homogenous cell population derived from hESCs [10]. These SCBEMs were cultured under hypoxic conditions (5 % O_2) with specific growth factors (BMP4, *glycogen synthase kinase 3* (GSK3) small inhibitor compound CHIR99021, and *fibroblast growth factor 2/beta* (FGF2)) to promote aggregation. These SCBEMs showed blastocoel-like cavity formation by day 3–4, and segregation of epiblast, hypoblast and trophectoderm compartments by day 6, also modelling post-implantation like morphologies in extended culture in ultra-low attachment well plates.

Kagawa et al. [7] generated static microwell-SCBEM of the blastocyst by aggregating triply inhibited (Hippo, TGF-β2 and *extracellular signal-regulated kinase* (ERK) pathways) naive hESCs in a microwell array, cultured under low oxygen conditions (5 % O_2). These SCBEMs resembled the B3–6/E5–7 late blastocysts morphologically and transcriptomically, featuring a cavitated structure and epiblast, hypoblast and trophectoderm lineage segregation by day 4. The microwell-SCBEMs of blastocyst could directionally attach to hormonally stimulated endometrial cells via the polar trophectoderm cells, as well remodel into post-implantation-like structures with pro-amniotic-like cavities, amnion, and extra-embryonic endoderm development under extended culture up to day 6. Interestingly, the trophoblasts expressed chorionic gonadotropin that was detectable with standard pregnancy tests.

Gao et al. demonstrated that naïve hESCs can spontaneously generate SCBEMs of blastocyst without exogenous differentiation factors, when aggregated in 3D (pyramidal microwells or suspension plates) and cultured with small molecule IM-12 instead of CHIR99021 as the GSK3 inhibitor in a defined multi-inhibitor naïve medium [6]. IM-12 upregulated the oxidative phosphorylation genes, promoting spontaneous SCBEM formation within 6 days. These SCBEMs resembled the day 5 blastocyst, with segregation of the epiblast, hypoblast and trophectoderm, and blastocyst-like morphology. Upon extended culture on matrix-coated 8-well slides, the spontaneous SCBEMs differentiated and showed key features of post-implantation development resembling day 14–19 embryos, including specification of mesoderm and endoderm populations, extraembryonic mesoderm, and primitive streak-like patterning.

The field quickly progressed to using the static aggregation approach to generate SCBEMs of post-implantation development [13–15,17,20], including gastrulation [24,27,32]. Ai et al. generated SCBEMs of post-implantation embryo, by assembling naive hESCs with 'signalling nest cells' (BMP4/SB431542 induced hESCs) in pyramidal microwells plates under normoxic (21 % O_2) conditions [13]. By day 8 of culture SCBEMs resembled day 13–14 natural embryos, mimicking key developmental landmarks of human peri-implantation embryos, including the formation of bilaminar disc, amniotic-like cavity, and yolk sac-like cavity, and extraembryonic mesoderm specification.

Further SCBEMs of post-implantation embryo [17,20] were subsequently published, both using pyramidal microwells. Weatherbee et al. [20,59] developed SCBEMs of post-implantation development through 3D aggregation of wild-type hESCs with two types of extraembryonic-like cells. These extraembryonic-like cells were generated via a transgene model with doxycycline-inducible overexpression of specific transcription factors: *GATA binding protein 6* (GATA6) and *SRY-box transcription factor 17* (SOX17) for hypoblast-like cells (iGATA6-Sox17) and *GATA binding protein 3* (GATA3) and *activating enhancer binding Protein 2 gamma* (AP2ɣ) for trophoblast-like cells (iGATA3-AP2ɣ). This SCBEM mimicked several aspects of post-implantation development including epiblast, hypoblast and trophectoderm segregation, and amnion, primordial germ cell, and extraembryonic mesoderm specification. In contrast, Pedroza et al. [17] created SCBEMs using only intermediate-state hESCs without genetic modification, leveraging precision-tuned starting cell phenotypes to enable self-organisation with only minimalist spontaneous differentiation medium. These SCBEMs resembled day 5–12 natural embryos, demonstrating epiblast-hypoblast segregation, anterior-posterior patterning of the epiblast, amnion-epiblast patterning, yolk sac-like cavity formation, primitive streak patterning, and transcriptomic confirmation of the post-implantation epiblast, amniotic ectoderm, mesoderm, early extra-embryonic endoderm cells.

Building on these foundational models, Okubo et al. recently developed SCBEMs that achieve bilaminar disc formation to specifically investigate hypoblast-epiblast interactions [15]. Their approach combined naïve hESCs or hiPSCs with hypoblast-like cells (nHyCs) that, unlike Weatherbee et al.'s transgene-based approach, could be generated through both genetic and non-genetic methods. These SCBEMs exhibited key post-implantation developmental features including bilaminar disc formation, epiblast anterior-posterior patterning, and specification of multiple cell types including amnion-like cells, primordial germ cell-like cells, and haematoendothelial progenitors.

Static microwell guided aggregation has been used to generate SCBEMs of more specific post-implantation developmental events, including a model by Hislop et al. [14] for studying early haematopoiesis, and a model by Gharibi et al. of amniogenesis [33]. Hislop et al. developed SCBEMs of haematopoiesis initiation by aggregating primed hiPSCs with inducible GATA6-expressing (iGATA6) hiPSCs in pyramidal microwells, generating SCBEMs resembling day 7–16 natural embryos [14]. The SCBEMs uniquely contained yolk sac-like tissue that recapitulated distinct waves of haematopoiesis with multiple blood cell lineages (erythroid, megakaryocyte, myeloid and lymphoid-like cells), in addition to bilaminar disc formation, anterior-posterior patterning of the epiblast, and amniotic cavity-like formation. Gharibi et al. [33] developed a post-gastrulation SCBEM, modelling weeks 2–4 post-gastrulation amnion and extra-embryonic tissue development. Using sequential BMP4 together with a small molecule WNT agonist treatment for 24 h each followed by aggregation in low-adherence plates, they generated fluid-filled amniotic sac-like structures with proper two-layered amnion (amniotic ectoderm and extraembryonic mesoderm) and yolk sac-like formation.

Static microwell guided aggregation has also supported the generation of various SCBEMs of gastrulation [24,27,32]. Moris et al. generated a SCBEM of gastrulation by pre-treating primed hESCs with a small molecule WNT agonist for 24 h, followed by 3D aggregation in low-adherence U-bottom plates [27]. These SCBEMs underwent axial elongation, with triploblastic patterning and gene expression patterns resembling day 20 natural embryos, including signatures of somitogenesis. More recent advances by Yuan et al. [32] and Liu et al. [24] introduced sequential two-stage media protocols for generating SCBEMs of gastrulation in pyramidal microwells. Yuan et al. aggregated hESCs or hiPSCs in a defined differentiation medium containing FGF2 and BMP4, to produce SCBEMs of gastrulation that recapitulated key morphological and transcriptional features of day 5–16 human embryos [32]. This included the formation of the bilaminar disc, primitive streak, amniotic-like cavity, yolk sac-like cavity, blood islets, as well as extraembryonic mesoderm and primordial germ cell specification. Liu et al. generated SCBEMs of gastrulation by aggregating hEPSCs in pyramidal microwells with sequential media changes from titrated hypoblast differentiation medium, to media supplemented with basement matrix and glucose [24]. This supported the development of a SCBEM, that, although lacked trophectoderm-like cells, recapitulated key gastrulation and post-implantation developmental events through early organogenesis, including bilaminar and trilaminar disc development, primitive streak formation, amniotic and yolk sac-like cavity formation, haematopoiesis, primordial germ cell specification, and initiation of neurulation, including neural tube formation.

2.4. Microfluidics

Microfluidic technologies enable precise control of morphogen signalling that static culture systems inherently lack (Fig. 2). Microfluidic gradients can be used to reproduce natural embryonic signals, in which cells respond to dynamic concentration gradients, fluid flows, and compartmentalised microenvironments that guide their fate decisions and tissue organisation. Static cultures fundamentally fail to recapitulate these dynamic conditions, particularly limiting the ability to model early patterning events where morphogen gradients orchestrate complex developmental programs.

Microfluidic devices achieve this control using miniaturised channel networks, typically fabricated in polydimethylsiloxane (PDMS) and bonded to glass or plastic substrates. These devices incorporate controlled flow systems using syringe or peristaltic pumps to generate stable morphogen gradients while applying physiologically relevant shear stresses [80]. This technology has enabled the generation of increasingly sophisticated human SCBEMs of gastrulation [25], amniogenesis [36,81], neurulation [40,43], and somitogenesis [45].

Manfrin et al. generated a microfluidic-SCBEM of gastrulation by engineering polyethylene glycol hydrogel barriers within microfluidic devices to create localised BMP4 sources with counteracting NOGGIN inhibitor gradients [31]. This broke the spatial limitations in non-microfluidic systems [25], to achieve more physiologically relevant axial organisation. Zheng et al. [36,81] developed a three-channel microfluidic system to generate microfluidic-SCBEM of amniogenesis. Their device was inspired by the agonist/antagonist morphogen pairs present during development, primarily BMP4 activation versus BMP/WNT inhibition, to differentiate hESC or hiPSC aggregates in basement matrix pockets. Rather than recapitulating only germ layer patterning as in the Manfrin study [25], this approach targeted specific morphological landmarks of human epiblast and amnion development, including epiblast lumenogenesis, formation of bipolar embryonic sacs, and specification of primordial germ cells and primitive streak cells. Importantly, this work revealed that amniotic ectoderm-like cells function as endogenous signalling centres that trigger gastrulation-like events, demonstrating how engineered microenvironments not only control initial patterning but can also uncover previously unknown developmental mechanisms.

Microfluidics has also supported the generation of neurulation-SCBEMs [40,43]. Rifes et al. developed microfluidic-SCBEMs of neural formation by exposing hESCs or hiPSCs on basement matrix -coated devices to GSK3 inhibitor gradients, creating corresponding WNT activation gradients along the tissue [40]. This approach generated patterned neural tissues with rostral-caudal organisation from forebrain to hindbrain, and captured key features of early neural development, including midbrain–hindbrain boundary formation and dorsal-ventral patterning. Xue et al. [43] took a more complex approach, combining micropatterning with microfluidics (Fig. 4B). Specifically, they combined microcontact-printed basement matrix islands with dual-axis morphogen gradients to create microfluidic-SCBEMs of the neural tube. This system enabled simultaneous control over both rostral-caudal and dorsal-ventral patterning, producing 3D tubular structures that recapitulated specific brain and spinal cord regions, as well as the development of dorsal-ventral patterned microfluidic forebrain-like structures with spatially segregated dorsal and ventral regions and layered apicobasal cellular organisations that mimic development of the human forebrain pallium and subpallium. This work revealed new insights into neural crest development and the contribution of neuromesodermal progenitors in human neurodevelopment, exemplifying how technical advances in SCBEM engineering can enable biological discovery.

Liu et al. developed a microfluidic-SCBEM of somitogenesis that combined microfluidics with biomaterial embedding [45]. By confining presomitic mesoderm cells derived from hESCs or hiPSCs within microfabricated trenches of basement matrix while imposing opposing retinoic acid and FGF8/WNT gradients, they triggered spontaneous, periodic somite formation with rostral-caudal patterning. The mechanical confinement provided essential boundary conditions absent in previous static microwell approaches, while the controlled gradients enabled systematic dissection of somitogenesis mechanisms, illustrating how microfluidics can integrate multiple developmental cues to achieve unprecedented model fidelity.

These studies collectively demonstrate that microfluidic technologies have evolved from simple gradient generators to sophisticated platforms capable of orchestrating complex developmental programs. By providing precise spatiotemporal control over both biochemical and biomechanical signals, microfluidics has enabled the generation of SCBEMs that not only recapitulate morphological features but can also serve as discovery platforms for understanding fundamental developmental mechanisms.

2.5. Dynamic microwell-initiated 3D aggregation

Building on the static microwell-guided 3D aggregation approach, dynamic microwell-initiated 3D aggregation has also been used to generate SCBEMs of post-implantation embryos (Fig. 2). In this context, this approach uses 3D aggregation in static systems, followed by dynamic culture technologies such as orbital shakers or roller culture bioreactor platforms to support longer term culture of SCBEMs.

For example, SCBEMs of axial elongation were generated by seeding pretreated adherent hiPSC colonies directly into well plates which generated 3D aggregates that were then able to be directly cultured on an orbital shaker and maintained for up to 40 days [39]. These SCBEMs showed axial elongation with mesoderm-endoderm boundary formation, primitive gut tube formation, as well as neurectoderm patterning, spinal cord dorsal-ventral patterning, and neural crest migration. Olmsted et al. expanded these SCBEMs to include further cardiac and neural tissue co-development, through temporal growth factor switching of angiocrine and pro-cardiogenic factors (vascular endothelial growth factor, ascorbic acid, increased FGF2) at 48 h post-aggregation, combined with dynamic orbital shaking culture [52]. These multi-chambered, contractile SCBEMs of cardiac crescent showed heart tube development, and critically, a progressive neuronal population of the cardiac region mimicking cardiac innervation.

Presently, one of the most advanced human SCBEMs of the post-implantation embryo leverages orbital shaking and roller culture technology to recapitulate features of the natural human embryo up to day 13–14 [16]. After day 8 post-aggregation in pyramidal microwells, this SCBEM demonstrated spatially organised morphogenesis of key embryonic and extraembryonic structures, including the bilaminar disc formation, amniotic-like sac, yolk-like sac and chorionic-like sac formation, amnion specification, connecting stalk formation, primordial germ cell and extraembryonic mesoderm specification, and uniquely, a surrounding trophoblast-like layer. These SCBEMs was generated through 3D aggregation of naïve hESCs, primitive endoderm-like/extraembryonic mesoderm-like cells, and trophectoderm-like cells using pyramidal microwell plates. From day 3 onwards, the aggregates were transferred to non-adherent 6-well plates on an orbital shaker, which prevented trophectoderm attachment to the well plates and enabled extended growth with improved morphology (Fig. 4D). Alternatively, from day 6 to 8, the researchers also employed an ex-utero roller culture platform - following orbital shaking from day 3 onward - but this approach did not lead to improvements in model development. Ex-utero roller culture platforms, or rotating bottle culture systems, were initially developed for prolonged ex-utero culture of natural mouse embryos [82], but were then adapted to support mouse SCBEMs (E5–8.5) [71], and later, human SCBEMs of post-implantation embryos [16]. These electronically controlled platforms enable more precise control of oxygen with high pressures, which when combined with the gentle continuous rolling movement and the optimised media formulations, enhances oxygenation and nutrient delivery to developing embryo

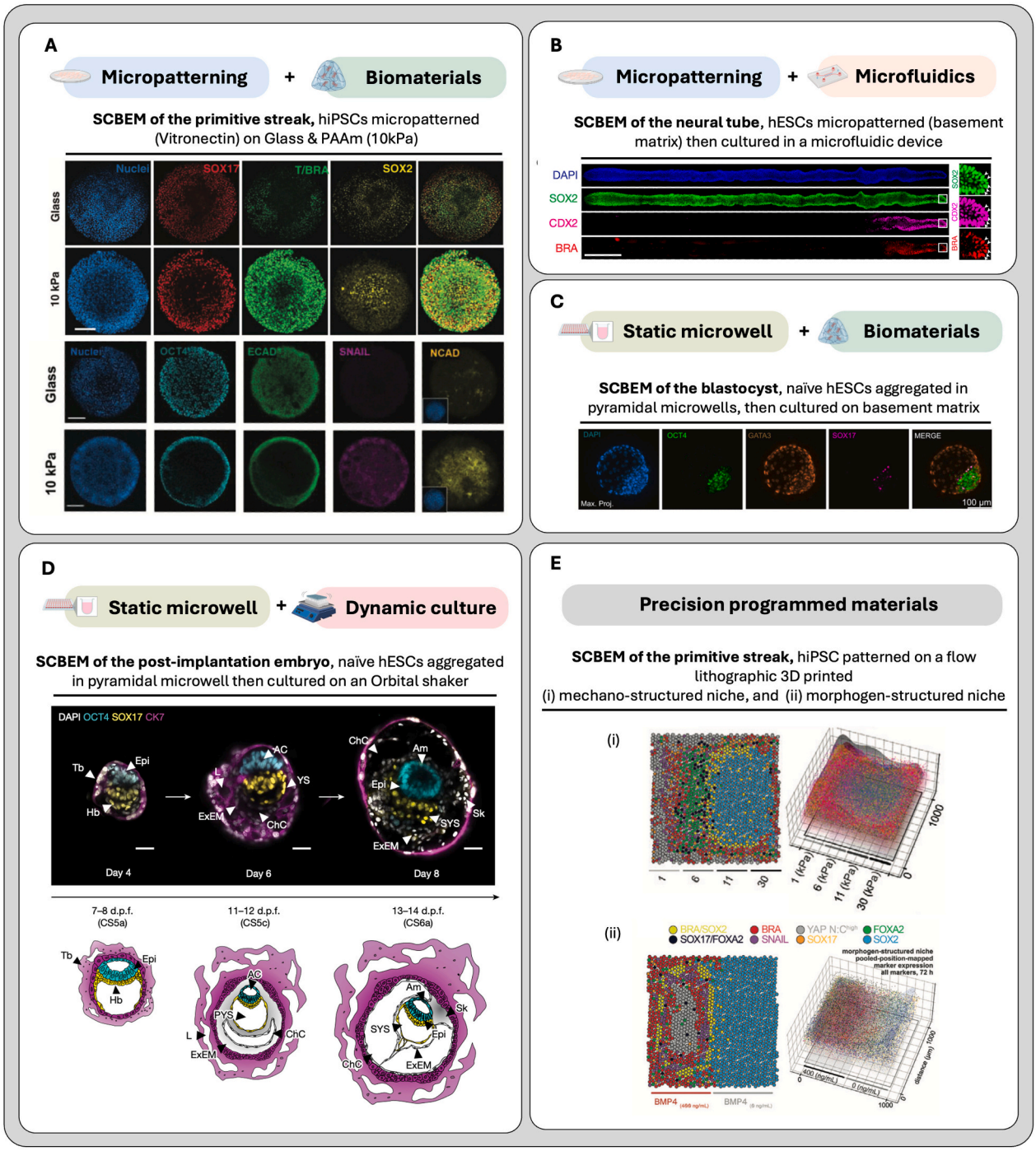


Fig. 4. Examples of hybrid engineering approaches for SCBEMs and next generation approaches. (A.) Micropatterning + Biomaterials - hiPSCs micropatterned (Vitronectin) on Glass and PAAm (10 kPa) – hiPSC colonies on *"patterned 10 kPa hydrogels inducted with BMP4 stained for SOX17, T/BRACHYURY and SOX2"*, and "*Immunofluorescence images for E-CAD, N-CAD, SNAIL, and OCT4 expression across colonies on glass and hydrogel patterns at 48 h"* [29]. (B.) Micropatterning + Microfluidics – "µNTLS" – hESCs micropatterned (Geltrex™) then cultured in a microfluidic device. *"Left, stitched confocal micrographs showing R–C patterned µNTLS on day 4 immunostained for SOX2, CDX2 and BRA. Right, zoom-in views of boxed regions as indicated. SOX2+CDX2+BRA+ cells are marked by arrowheads."* [43]. (C) Static microwell guided 3D aggregation + Biomaterials – "3D cultured blastoid" – "*Maximum projection IF confocal imaging of a WIBR3 OCT4-GFP blastoid at day 7. OCT4 indicates blastoid bEPI, GATA3 marks surrounding blastoid bTE, and SOX17 marks bPE. Scale bar, 100 µm"* [8]. (D) Static microwell guided 3D aggregation + Dynamic culture – "SEMs" – *"Right to left, representative immunofluorescence images (top) and schematics (bottom) of SEMs from days 4, 6 and 8 showing OCT4, SOX17, CK7 and nuclei (DAPI). d.p.f. values are approximate"* [16]. (E) Precision programmed materials [56] – (i) Germ layer differentiation of hiPSCs on a flow lithographic 3D printed mechano-structured niche (1 kPa, 6 kPa, 11 kPa, 30 kPa), showing that softer regions promote mesodermal and endodermal differentiation, and stiffer regions promote ectodermal differentiation. (ii) Patterning of hiPSCs on a flow lithographic 3D printed morphogen-structured niche (BMP4), showing mesodermal cells surrounded by endodermal cells in BMP4 patterned regions, and ectodermal cells in the regions without BMP4. (A) [29], © 2022 The Authors. Advanced Science published by Wiley-VCH GmbH; (B) [43], © 2024 The Authors. Nature published by Springer Nature BV; (C) [8], © 2023 The Authors. Cell Stem Cell published by Elsevier Science & Technology Journals; (D) [16], © 2023 The Authors. Nature published by Springer Nature BV; (E) [56], © 2023 The Authors. Advanced Science published by Wiley-VCH GmbH.

models. However, in the case of Oldak's post-implantation-SCBEM, there was no improvements in model formation when using the roller culture platform over just the orbital shaker, potentially because the 14-day culture period was insufficient to observe any long-term benefits.

Most recently, Chen et al. used a simplified approach to generate SCBEMs of the post-implantation embryo [21]. Using STAT3 activation combined with TGF-β2 inhibition of hESCs and hiPSCs, they generated SACs (STAT3-activated cells), multi-lineage cell populations containing naïve epiblast-like cells as well as hypoblast-, trophectoderm-, and extraembryonic mesoderm-like cells. Aggregation of SACs in pyramidal microwells followed by orbital shaker culture enabled their self-organisation into post-implantation SCBEMs resembling weeks 2–3 (Carnegie stage 5–7) embryos within 6 days of culture.

3. Future scope

With their rapidly advancing physiological accuracy, SCBEMs are emerging as a compelling alternative to using natural embryonic materials in developmental biology studies. Particularly in the case of humans, where the economic and ethical considerations are paramount, we expect SCBEMs to become a predominant model for studies in human development. However, it should be noted that SCBEMs are presently unable to fully recapitulate the numerous complex functions of their natural counterparts. This limitation partly reflects the recency of culture methods, that lack comprehensive management of their metabolic functions, alongside cellularity and architectural formation, including the corresponding histo- and morpho-genetic processes that characterise these attributes – all attributes that may be greatly enhanced through use of engineering principles.

This section explores future directions for engineering approaches to advance SCBEM models, including the relevance of engineering technologies in advancing physiological accuracy, standardisation efforts necessary to ensure robustness and reproducibility, and the evolving ethical landscape surrounding these increasingly sophisticated models and the use of engineering perturbation in this context.

3.1. Current limitations and emerging engineering solutions

While the field has progressed considerably through media optimisation and various technological approaches outlined in previous sections (Fig. 3, Table 1), several limitations have restricted the potential of current SCBEMs. In the below subsections we explore these and discuss their technological resolution.

3.1.1. Overcoming metabolic constraints and vascular solutions

A critical bottleneck in advancing SCBEMs is the diffusive and metabolic constraints that limit their size. Most current models are statically cultured and limited by an inability to acquire sufficient energy and nutrients developing central hypoxia and "dead cores" [16,82]. While the quantitative limits remain unmeasured in human SCBEMs, neural organoids show that the critical oxygen threshold for cell survival is ~0.04 mol·m^{-3}, which can be partially overcome with perfusion culture (for example hypoxia is reduced ~1.8 % vs ~6.4 % comparing perfusion bioreactors with orbital shakers [83].

It is notable that comparison with organoids is precarious, since embryonic tissues are some of the most metabolically demanding, with classic measurements show a ~5-fold rise in CO_2 production from glucose at fertilisation and ~100-fold increase across the first 5 days of mouse preimplantation development, and single-embryo O_2-consumption measurements also climb over cleavage-to-blastocyst stages [84,85]. Such high metabolic demands underscores the need to keep O_2 and nutrient delivery commensurate with growth, and reinforces conclusions that human SCBEMs are metabolically limited [16].

Natural embryonic development relies on vasculature - an efficient metabolic interface that circulates nutrients and removes waste products through advection rather than diffusion alone. As SCBEMs increase in complexity and size, diffusion-based nutrient transport becomes increasingly insufficient, resulting in metabolic stress and developmental arrest. While high-glucose media and dynamic culture platforms such as orbital shakers and roller culture platforms have been employed to improve nutrient delivery, these approaches create unphysiological conditions that may impair development. This represents a fundamental constraint that media optimisation and dynamic culture alone cannot overcome.

Engineered vascular networks represent perhaps the most promising solution to this bottleneck – this approach is, after all, how natural mammalian embryos are supported. Several approaches show potential for integration with SCBEMs including 3D printing and sacrificial templating approaches [86,87], such as creating perfusable channels using sacrificial materials that can be removed after embedding cells in hydrogels [88], or 3D printing techniques with multi-scale vasculature [36,89–91]. Alternatively, SCBEMs might leverage insights from recent advances in vascularised organoid or assembloid technology [92]. Thus far, this strategy has been successfully deployed for kidney organoids where culture in microfluidic chips with low flows enhanced vascularisation, leading to increased oxygenation and waste removal [93]. A vascular approach would also support the stationary culture of SCBEMs, which is advantageous as they would then be amenable to more constant observation. Based on these successes, we encourage exploring SCBEMs culture in perfusion bioreactor systems with interfacial flows in the ~10^{-3}–10^{-2} dyn·cm^{-2} regime during acclimation and up to ~0.03 dyn·cm^{-2}, providing a proto-maternal interface can be established. Studies can benchmark perfusion culture against an orbital-shaker control to quantify reductions in low-O_2 fraction (e.g., hypoxia dyes, apoptosis markers) and gains in lineage maturation.

3.1.2. Precision control of signalling

Current SCBEM generation methods primarily rely on globally administered exogenous differentiation stimuli. While this approach has yielded the most developmentally competent models to date, it presents fundamental limitations for reproducibility and precise patterning. Since developmental processes involve diffusion, gradient formation, and signal transduction, they inherently exhibit chaotic dynamics, where minor variations in initial conditions can produce dramatically different outcomes [94,95]. This stochasticity generates SCBEMs with substantial structural variability and often non-recapitulative architectures, limiting both reproducibility and physiological relevance.

Engineering approaches offer solutions through precise spatiotemporal control of developmental signals. Microfluidic platforms can generate stable, reproducible cellular scale morphogen gradients that recapitulate embryonic signalling environments [25]. These systems enable orthogonal delivery of multiple morphogens with independent spatial control, which is crucial for achieving complex patterning events. Complementing these approaches, optogenetic and chemogenetic control systems provide unprecedented temporal precision, allowing targeted activation of specific signalling pathways at defined developmental stages [96]. Alternatively, precision programmed materials represent another promising strategy for providing spatiotemporally controlled signalling without continuous external intervention [56,97,98] (Fig. 4E). For instance, setting initial matrix conditions to align with key developmental programs can provide the context in which endogenous morphogen gradients are initiated and propagated in a developmentally relevant fashion [29] (Fig. 4 A). Materials design to render these scaffolds dynamic to changing signal gradients is an exciting prospect, essentially encoding "dynamic reciprocity" into a system to align with developmental stages, i.e. stimuli triggered "softening" or "stiffening", change in architecture, sequestered growth factor release, etc., [99,100]. Viewed through the spatial lens, these tools progress from implicit mesoscale programming (aggregate size, gel thickness/stiffness) to explicit micro-scale programming (microfluidic chip gradients, photo-addressable chemistries), letting investigators dial in the spatial resolution that matches the native process under study.

Overall, by integrating these technologies, researchers can transition from stochastic self-organisation toward deterministic guidance, potentially achieving more reproducible and physiologically relevant SCBEMs.

3.1.3. Integration of extraembryonic tissues and maternal interfaces

A significant limitation of current SCBEMs is their development in isolation from maternal tissues, which fails to recapitulate the critical bidirectional signalling that occurs at the maternal-foetal interface. Several promising engineering strategies are emerging to address this challenge, including microfluidic platforms that integrate SCBEMs with engineered decidual tissue models to recreate implantation interfaces [101], and the co-culture of blastocyst-SCBEMs with hormone-responsive endometrial organoids that emulate in vivo architecture [7,102]. These co-culture systems have successfully recapitulated critical implantation stages, including apposition, adhesion, and invasion [7]. More advanced SCBEMs have also begun to incorporate hypoblast-like lineages alongside embryonic tissues [17], and some can develop to stages equivalent to early organogenesis while maintaining both embryonic and extraembryonic components [24].

We suggest that future engineering approaches should focus on refining biomaterial-based decidua mimetics. Materials-based approaches offer significant advantages through their relative simplicity compared to complex organoid and assembloid type systems, allowing for rapid iteration and systematic exploration of morphogenetic signals and environmental factors. This simplicity aligns with the other aspects of SCBEM development, where carefully defined initial conditions and globally administered signals produce sophisticated developmental outcomes. Further, biomaterials-based decidua offers straightforward engineering of void space or vasculature to support the metabolic needs of the SCBEM. By integrating these maternal interface elements, future SCBEMs could better capture the complex bidirectional signalling at the implantation site, potentially advancing our understanding of pregnancy disorders and implantation failure while providing more physiologically relevant models for reproductive and developmental research.

3.2. Standardisation of SCBEMs through engineering approaches

As SCBEMs advance in complexity (Fig. 3), standardisation has emerged as a critical need to ensure reproducibility, comparability, and ethical oversight. Martinez Arias et al. recently proposed a comprehensive criteria for standardising SCBEMs, emphasising the assessment of both efficiency (reproducibility of generation) and fidelity (similarity to natural embryos) [57]. This framework includes quantifiable attributes such as morphological characteristics, transcriptional profiles, spatial organisation, and developmental timing - all aspects that can be enhanced through engineering approaches. Below we expand on this standardisation effort as it relates to relevant engineering approaches.

Martinez Arias et al. 2024 proposed a standardised nomenclature where specific features of embryogenesis being modelled are added as suffixes (e.g., “SCBEM of gastrulation”) [57]. We expand on this, suggesting that nomenclature could be extended to include engineering/technological or computational prefixes/suffixes when appropriate. This has been used throughout the above (i.e. “microfluidic-SCBEM of somitogenesis”). As the field matures, these standardisation efforts will be essential for generating robust and reproducible models with consistent quality attributes, allowing meaningful cross-laboratory comparisons and accelerating translation to therapeutic applications.

The implementation of standardised technology platforms represents a crucial step toward achieving reproducible SCBEMs. Automated production systems [103] provide a modular, robotic platform for high-throughput, standardised cell line generation with significantly reduced line-to-line variation compared to manual methods. Similar approaches have proven successful in the more mature field of CAR-T cell manufacturing, where closed-system bioreactors with precise control of culture parameters have dramatically improved batch consistency [104,105]. For SCBEMs, standardised microfluidic platforms that provide controlled gradients of morphogens represent another promising technology, enabling reproducible spatiotemporal signalling essential for developmental patterning [36]. Standardised biomaterials and matrix mimics with defined mechanical and biochemical properties are also being developed to ensure consistent cell-matrix interactions across experiments [42,56]. Together, these technological standardisation efforts aim to transform SCBEM generation from an art dependent on researcher experience to a robust engineering process with predictable outcomes.

Beyond physical engineering platforms, computational modelling represents a powerful approach for standardising and predicting SCBEM development, bringing mathematical rigor to inherently variable biological systems. Machine learning algorithms trained on high-content imaging data offer another standardisation approach, as demonstrated by [106], where deep neural networks reduced morphological profile variation across batches and identified disease-specific signatures with high reproducibility. Similar approaches by [107,108] have developed frameworks to analyse cellular organisation from imaging data, with the latter generating a comprehensive dataset of over 200,000 live cells spanning 25 key cellular structures and creating analytical methods to quantify organisational features despite inherent biological variability. These approaches are particularly relevant for SCBEMs, where proper spatial organisation is a critical quality attribute. When integrated with automated production systems, these computational approaches enable real-time monitoring with feedback control, allowing for dynamic adjustment of engineering parameters during SCBEM development [103].

3.3. Ethical considerations

3.3.1. Current ethical frameworks and engineering challenges

Running parallel to traditional bioethics frameworks that focus primarily on the moral status of embryos themselves, there have been several major initiatives focused on SCBEM research. The *International Society for Stem Cell Research Guidelines 2021* provided an early framework that categorised SCBEMs into ‘integrated’ and ‘non-integrated’ types, establishing different levels of oversight based on model complexity [2]. However, subsequent scientific advances have already challenged these classifications, with models previously considered ‘non-integrated’ demonstrating developmental competence far beyond what was initially anticipated [16]. Rivron et al. later offered a more conceptual framework centred on developmental competence and potential, proposing a refined legal definition of embryos and suggesting ‘tipping points’ based on two indirect tests that would determine when SCBEMs should be regulated similar to natural embryos [109]. A recent standard by the *Nuffield Council on Bioethics* identifies regulatory gaps and recommends a three-stage governance approach, proposing that SCBEMs should be considered distinct from embryos while establishing clear boundaries including an upper threshold at Carnegie Stage 23 [110]. And finally, the *Cambridge Reproduction Code of Practice 2024* provides detailed practical guidance for researchers and funders, establishing an Oversight Committee and advocating for case-by-case review rather than fixed developmental limits [111].

In contrast, engineering SCBEMs faces unique ethical challenges as their design decisions directly influence the developmental potential and capabilities of these models. Specific engineering ethics must grapple with “moral status by design” - where technical choices deliberately enhance or limit certain capabilities. This is particularly important as engineering approaches have become a critical component of supporting late-stage mouse SCBEMs, with increasing developmental competence: likely to go hand-in-hand with more advanced engineering tools. Engineers and scientists must understand that design choices themselves have moral dimensions – engineered features might fundamentally change the moral evaluation of SCBEMs, where the

technological design can aim to faithfully recapitulate development, deliberately limit development, or even extend it beyond what naturally occurs [112].

3.3.2. *Technical boundaries and enabling technologies*

We propose that use of these enabling technologies outlined in Table 2 warrant additional specific ethical considerations – including whether or not these technologies themselves should be modified with an appropriate predetermined developmental constraint that prevent unintended developmental progression. This framework provides actionable guidance that moves beyond general principles to specific implementation mechanisms tailored to the unique challenges of engineered developmental systems.

Further, future efforts, particularly those by relevant governance [2,110,111], should consider whether appropriate classification of SCBEM research can additionally be based on the specific enabling technologies employed, in addition to other classifications schemes, such as morphological features.

Table 2
| Technical boundaries, enabling technologies, and ethical implications in SCBEM engineering.

Technical Boundary	Enabling Technologies	Contributing Fields	Oversight Considerations	Ethical Implications
Perfused metabolic support, Vascular-like	Engineered vasculature [86–90] Perfusable microfluidic platforms [36]; Vascularised organoid integration [92]; Biomimetic oxygen carriers	Organ-on-chip engineering; Vascular biology; Biomaterials science	**Qualitative trigger:** metabolic independence from diffusion-limited culture **Framework status:** Exceeds 14-day rule assumptions [2]; Gap identified [110]	Extended developmental potential beyond current limitations; models potentially crossing 14-day equivalent
Neural system complexity	Advanced neural organoid integration; Electrical stimulation systems; Functional neural circuit formation	Neuroscience; Neuroengineering; Brain-on-chip technologies	**Qualitative trigger:** High complexity/structural similarity with animals **Framework status:** Addressed by sentience considerations in [110]; Case-by-case review recommended [111]	Potential for rudimentary sensory processing; questions about sentience thresholds and consciousness
Maternal-embryonic interface	Endometrial organoid integration [7,101,103]; Artificial decidua systems; Hormonal regulation platforms	Reproductive biology; Microfluidic co-culture [97]; Endocrinology	**Qualitative trigger:** Bidirectional signalling recapitulates implantation **Framework status:** Currently unaddressed	Models of implantation failure and pregnancy disorders; potential therapeutic applications raising regulatory questions
Extraembryonic membranes	Engineered placental barriers; Functional trophoblast systems [7]; Gas exchange membranes;, Ex-utero culture platforms [16,71,82]	Placental biology; Barrier tissue engineering; Membrane technology	**Qualitative trigger:** Gas exchange capacity enabling autonomous metabolic support **Framework status:** Integrated models require enhanced oversight [2]; Three-stage governance approach [110]	Creation of systems with potential for extended ex-utero development; artificial womb implications
Scalable manufacturing	Automated bioreactor systems [103]; High-throughput fabrication; AI-guided quality control [106–108]; GMP-compliant production [104,105]	Bioprocess engineering; Cultivated meat technology; Factory automation	**Qualitative trigger:** Production systems enabling standardised, reproducible generation at scale **Framework status:** Commercial applications require regulatory frameworks [110]	Mass production possibilities raising commodification concerns; potential commercial applications requiring regulatory frameworks
Genetic manipulation	Precise developmental gene control; Inducible developmental pathways [96]; Synthetic developmental circuits	Synthetic biology; Gene editing technologies; Systems biology	**Qualitative trigger:** Modifications that alter developmental potential or trajectory **Framework status:** "Moral status by design" considerations [112]; Enhanced oversight for integrated models [2]	Enhanced or altered developmental potential; Questions about human enhancement versus therapy
Cross-species integration	Human-animal chimeras for organ development; Interspecies blastocyst complementation; Engineered species barriers	Xenotransplantation; Chimera biology; Comparative embryology	**Qualitative trigger:** Integration enabling human development in non-human systems **Framework status:** Addressed separately by chimera guidelines [2]; Distinct ethical considerations [111]	Questions about species identity and appropriate boundaries; applications for organ development
Computational guidance	Digital twin embryo models [103,106]; AI-guided developmental optimisation; Machine learning prediction systems [107,108]	Computational biology; Artificial intelligence; Systems modelling	**Qualitative trigger:** Prediction that guides or determines developmental outcomes **Framework status:** Currently unaddressed	Predictive engineering of developmental outcomes; questions about determinism in development
Self-limiting mechanisms	Engineered developmental checkpoints; Inducible apoptotic systems; Oxygen/nutrient-dependent constraints	Synthetic developmental biology; Biocontainment engineering; Safety systems design	**Qualitative trigger:** Technical safeguards that deliberately limit developmental potential by design **Framework status:** Proposed as governance mechanism [112]; Anticipatory governance approach	Technical safeguards that deliberately limit developmental potential; governance through design

3.4. Conclusion

The most successful engineering approach to advance SCBEMs will likely prioritise simplicity and focus on fundamental physiological requirements, where a developmentally specific context is presented to cultured cell populations. That is, provide systems where differentiation and morphogenesis proceed as dictated by the inherent biology of the cells, nurtured through biomimetic guidance cues. High-fidelity perfusion bioreactor systems represent a primary candidate for introducing maternal signalling while addressing both metabolic constraints and diffusion limitations that currently impede SCBEM development (such as precision fabricated biodevices [56,89,113]. These systems would enable the delivery of nutrients and removal of waste products via advection rather than diffusion alone, more closely mimicking natural mammalian embryonic interface.

Following establishment of robust perfusion platforms, incremental integration of additional complexity becomes feasible. This stepwise approach might first incorporate controlled temporal delivery of maternal signalling molecules through the perfusion network, followed by potential integration of maternal tissue interfaces within the bioreactor system – pending the necessity of these components as established through experimentation. Recent advances in synthetic implantation models suggest the feasibility of such integration while maintaining system stability.

Engineering approaches offer dual advantages for developmental biology. First, they enable systematic reconstruction of embryonic environments, adding defined components sequentially to identify sufficient conditions for specific developmental processes. Second, and perhaps more valuably, they facilitate controlled deconstruction of developmental systems through parametric perturbation studies. Unlike observational approaches in natural embryos, engineered platforms permit isolated manipulation of individual variables within complex developmental environments, enabling rigorous testing of mechanistic hypotheses through reductionist experimentation. By balancing these reconstructive and deconstructive approaches, engineering methods offer unique opportunities to dissect the design principles underlying human embryogenesis while advancing toward increasingly physiologically relevant models for both basic and translational research.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work was supported through funding from a NSW Health Cardiovascular Research Capacity Program Senior Researcher Grant (K.A. K.), and the National Cancer Institute of the National Institutes of Health, United States Grant R01CA251443 (K.A.K.). E.G.L acknowledges scholarship support from the Australian Government Research Training Program, the Australian Academy of Technological Sciences & Engineering Elevate Program, and the Baxter Family Postgraduate Scholarship.

Data availability

No data was used for the research described in the article.

References

[1] J. Fu, A. Warmflash, M.P. Lutolf, Stem-cell-based embryo models for fundamental research and translation, Nat. Mater. 20 (2) (2021) 132–144, https://doi.org/10.1038/s41563-020-00829-9.
[2] ISSCR Guidelines for Stem Cell Research and Clinical Translation, International Society for Stem Cell Research, May 2021.
[3] J. Wu, J. Fu, Toward developing human organs via embryo models and chimeras, Cell 187 (13) (2024) 3194–3219, https://doi.org/10.1016/j.cell.2024.05.027.
[4] X. Xue, Y. Liu, J. Fu, Bioengineering embryo models, Nat. Rev. Bioeng. 3 (1) (2024) 11–29, https://doi.org/10.1038/s44222-024-00241-x.
[5] Y. Fan, et al., Generation of human blastocyst-like structures from pluripotent stem cells, Cell Discov. 7 (1) (2021) 81, https://doi.org/10.1038/s41421-021-00316-8.
[6] M. Guo, et al., Self-renewing human naïve pluripotent stem cells dedifferentiate in 3D culture and form blastoids spontaneously, Nat. Commun. 15 (1) (2024) 668, https://doi.org/10.1038/s41467-024-44969-x.
[7] H. Kagawa, et al., Human blastoids model blastocyst development and implantation, Nature 601 (7894) (2022) 600–605, https://doi.org/10.1038/s41586-021-04267-8.
[8] R.M. Karvas, et al., 3D-cultured blastoids model human embryogenesis from pre-implantation to early gastrulation stages, Cell Stem Cell 30 (9) (2023) 1148–1165.e7, https://doi.org/10.1016/j.stem.2023.08.005.
[9] X. Liu, et al., Modelling human blastocysts by reprogramming fibroblasts into iBlastoids, Nature 591 (7851) (2021) 627–632, https://doi.org/10.1038/s41586-021-03372-y.
[10] B. Sozen, V. Jorgensen, B.A.T. Weatherbee, S. Chen, M. Zhu, M. Zernicka-Goetz, Reconstructing aspects of human embryogenesis with pluripotent stem cells, Nat. Commun. 12 (1) (2021) 5550, https://doi.org/10.1038/s41467-021-25853-4.
[11] A. Yanagida, D. Spindlow, J. Nichols, A. Dattani, A. Smith, G. Guo, Naive stem cell blastocyst model captures human embryo lineage segregation, Cell Stem Cell 28 (6) (2021) 1016–1022.e4, https://doi.org/10.1016/j.stem.2021.04.031.
[12] L. Yu, et al., Blastocyst-like structures generated from human pluripotent stem cells, Nature 591 (7851) (2021) 620–626, https://doi.org/10.1038/s41586-021-03356-y.
[13] Z. Ai, et al., Dissecting peri-implantation development using cultured human embryos and embryo-like assembloids, Cell Res. 33 (9) (2023) 661–678, https://doi.org/10.1038/s41422-023-00846-8.
[14] J. Hislop, et al., Modelling post-implantation human development to yolk sac blood emergence, Nature 626 (7998) (2024) 367–376, https://doi.org/10.1038/s41586-023-06914-8.
[15] T. Okubo, et al., Hypoblast from human pluripotent stem cells regulates epiblast development, Nature 626 (7998) (2024) 357–366, https://doi.org/10.1038/s41586-023-06871-2.
[16] B. Oldak, et al., Complete human day 14 post-implantation embryo models from naive ES cells, Nature (2023), https://doi.org/10.1038/s41586-023-06604-5.
[17] M. Pedroza, et al., Self-patterning of human stem cells into post-implantation lineages, Nature 622 (7983) (2023) 574–583, https://doi.org/10.1038/s41586-023-06354-4.
[18] M. Simunovic, et al., A 3D model of a human epiblast reveals BMP4-driven symmetry breaking, Nat. Cell Biol. 21 (7) (2019) 900–910, https://doi.org/10.1038/s41556-019-0349-7.
[19] M. Simunovic, E.D. Siggia, A.H. Brivanlou, In vitro attachment and symmetry breaking of a human embryo model assembled from primed embryonic stem cells, Cell Stem Cell 29 (6) (2022) 962–972.e4, https://doi.org/10.1016/j.stem.2022.05.001.
[20] B.A.T. Weatherbee, et al., Pluripotent stem cell-derived model of the post-implantation human embryo, Nature 622 (7983) (2023) 584–593, https://doi.org/10.1038/s41586-023-06368-y.
[21] C. Chen, et al., Signaling reprogramming via Stat3 activation unravels high-fidelity human post-implantation embryo modeling, Cell Stem Cell 32 (10) (2025) 1528–1544.e10, https://doi.org/10.1016/j.stem.2025.08.011.
[22] S. Chhabra, L. Liu, R. Goh, X. Kong, A. Warmflash, Dissecting the dynamics of signaling events in the BMP, WNT, and NODAL cascade during self-organized fate patterning in human gastruloids, PLoS Biol. 17 (10) (2019) e3000498, https://doi.org/10.1371/journal.pbio.3000498.
[23] F. Etoc, et al., A balance between secreted inhibitors and edge sensing controls Gastruloid self-organization, Dev. Cell 39 (3) (2016) 302–315, https://doi.org/10.1016/j.devcel.2016.09.016.
[24] L. Liu, et al., Modeling post-implantation stages of human development into early organogenesis with stem-cell-derived peri-gastruloids, Cell 186 (18) (2023) 3776–3792.e16, https://doi.org/10.1016/j.cell.2023.07.018.
[25] A. Manfrin, et al., Engineered signaling centers for the spatially controlled patterning of human pluripotent stem cells, Nat. Methods 16 (7) (2019) 640–648, https://doi.org/10.1038/s41592-019-0455-2.
[26] I. Martyn, T.Y. Kanno, A. Ruzo, E.D. Siggia, A.H. Brivanlou, Self-organization of a human organizer by combined Wnt and Nodal signalling, Nature 558 (7708) (2018) 132–135, https://doi.org/10.1038/s41586-018-0150-y.
[27] N. Moris, et al., An in vitro model of early anteroposterior organization during human development, Nature 582 (7812) (2020) 410–415, https://doi.org/10.1038/s41586-020-2383-9.
[28] J.M. Muncie, N.M.E. Ayad, J.N. Lakins, X. Xue, J. Fu, V.M. Weaver, Mechanical tension promotes formation of gastrulation-like nodes and patterns mesoderm specification in human embryonic stem cells, Dev. Cell 55 (6) (2020) 679–694.e11, https://doi.org/10.1016/j.devcel.2020.10.015.
[29] P. Srivastava, et al., Defined microenvironments trigger in vitro gastrulation in human pluripotent stem cells, Adv. Sci. 10 (5) (2023) 2203614, https://doi.org/10.1002/advs.202203614.
[30] M. Tewary, et al., A stepwise model of reaction-diffusion and positional-information governs self-organized human peri-gastrulation-like patterning, Development (2017) dev.149658, https://doi.org/10.1242/dev.149658.

[31] A. Warmflash, B. Sorre, F. Etoc, E.D. Siggia, A.H. Brivanlou, A method to recapitulate early embryonic spatial patterning in human embryonic stem cells, Nat. Methods 11 (8) (2014) 8, https://doi.org/10.1038/nmeth.3016.

[32] G. Yuan, et al., Establishment of a novel non-integrated human pluripotent stem cell-based gastruloid model, June 28 (2023), https://doi.org/10.1101/2023.06.28.546720.

[33] B. Gharibi, et al., Post-gastrulation amnioids as a stem cell-derived model of human extra-embryonic development, Cell (2025), https://doi.org/10.1016/j.cell.2025.04.025 p. S0092867425004581.

[34] Y. Shao, et al., Self-organized amniogenesis by human pluripotent stem cells in a biomimetic implantation-like niche, Nat. Mater. 16 (4) (2017) 419–425, https://doi.org/10.1038/nmat4829.

[35] Y. Shao, K. Taniguchi, R.F. Townshend, T. Miki, D.L. Gumucio, J. Fu, A pluripotent stem cell-based model for post-implantation human amniotic sac development, Nat. Commun. 8 (1) (2017) 208, https://doi.org/10.1038/s41467-017-00236-w.

[36] Y. Zheng, et al., Controlled modelling of human epiblast and amnion development using stem cells, Nature 573 (7774) (2019) 421–425, https://doi.org/10.1038/s41586-019-1535-2.

[37] T. Haremaki, J.J. Metzger, T. Rito, M.Z. Ozair, F. Etoc, A.H. Brivanlou, Self-organizing neuruloids model developmental aspects of Huntington's disease in the ectodermal compartment, Nat. Biotechnol. 37 (10) (2019) 1198–1208, https://doi.org/10.1038/s41587-019-0237-5.

[38] E. Karzbrun, et al., Human neural tube morphogenesis in vitro by geometric constraints, Nature 599 (7884) (2021) 268–272, https://doi.org/10.1038/s41586-021-04026-9.

[39] Z.T. Olmsted, J.L. Paluh, Co-development of central and peripheral neurons with trunk mesendoderm in human elongating multi-lineage organized gastruloids, Nat. Commun. 12 (1) (2021) 3020, https://doi.org/10.1038/s41467-021-23294-7.

[40] P. Rifes, et al., Modeling neural tube development by differentiation of human embryonic stem cells in a microfluidic WNT gradient, Nat. Biotechnol. 38 (11) (2020) 1265–1273, https://doi.org/10.1038/s41587-020-0525-0.

[41] G. Sahni, et al., A micropatterned human-specific Neuroepithelial tissue for modeling gene and Drug-induced neurodevelopmental defects, Adv. Sci. 8 (5) (2021) 2001100, https://doi.org/10.1002/advs.202001100.

[42] X. Xue, et al., Mechanics-guided embryonic patterning of neuroectoderm tissue from human pluripotent stem cells, Nat. Mater. 17 (7) (2018) 633–641, https://doi.org/10.1038/s41563-018-0082-9.

[43] X. Xue, et al., A patterned human neural tube model using microfluidic gradients, Nature 628 (8007) (2024) 391–399, https://doi.org/10.1038/s41586-024-07204-7.

[44] Y. Zheng, et al., Dorsal-ventral patterned neural cyst from human pluripotent stem cells in a neurogenic niche, Sci. Adv. 5 (12) (2019) eaax5933, https://doi.org/10.1126/sciadv.aax5933.

[45] Y. Liu, et al., A human pluripotent stem cell-based somitogenesis model using microfluidics, Cell Stem Cell 31 (8) (2024) 1113–1126.e6, https://doi.org/10.1016/j.stem.2024.06.004.

[46] Y. Miao, et al., Reconstruction and deconstruction of human somitogenesis in vitro, Nature 614 (7948) (2023) 500–508, https://doi.org/10.1038/s41586-022-05655-4.

[47] M. Sanaki-Matsumiya, et al., Periodic formation of epithelial somites from human pluripotent stem cells, Nat. Commun. 13 (1) (2022) 2325, https://doi.org/10.1038/s41467-022-29967-1.

[48] Y. Yamanaka, et al., Reconstituting human somitogenesis in vitro, Nature 614 (7948) (2023) 509–520, https://doi.org/10.1038/s41586-022-05649-2.

[49] S. Gribaudo, et al., Self-organizing models of human trunk organogenesis recapitulate spinal cord and spine co-morphogenesis, Nat. Biotechnol. 42 (8) (2024) 1243–1253, https://doi.org/10.1038/s41587-023-01956-9.

[50] N. Hamazaki, et al., Retinoic acid induces human gastruloids with posterior embryo-like structures, Nat. Cell Biol. 26 (10) (2024) 1790–1803, https://doi.org/10.1038/s41556-024-01487-8.

[51] Y.I. Yaman, S. Ramanathan, Controlling human organoid symmetry breaking reveals signaling gradients drive segmentation clock waves, Cell 186 (3) (2023) 513–527.e19, https://doi.org/10.1016/j.cell.2022.12.042.

[52] Z.T. Olmsted, J.L. Paluh, A combined human gastruloid model of cardiogenesis and neurogenesis, iScience 25 (6) (2022) 104486, https://doi.org/10.1016/j.isci.2022.104486.

[53] Blastocyst culture and transfer in clinically assisted reproduction: a committee opinion, Fertil. Steril. 110 (7) (2018) 1246–1252, https://doi.org/10.1016/j.fertnstert.2018.09.011.

[54] A. Deglincerti, G.F. Croft, L.N. Pietila, M. Zernicka-Goetz, E.D. Siggia, A. H. Brivanlou, Self-organization of the in vitro attached human embryo, Nature 533 (7602) (2016) 251–254, https://doi.org/10.1038/nature17948.

[55] M.N. Shahbazi, et al., Self-organization of the human embryo in the absence of maternal tissues, Nat. Cell Biol. 18 (6) (2016) 700–708, https://doi.org/10.1038/ncb3347.

[56] P.L.H. Newman, et al., Programming of multicellular patterning with Mechano-chemically microstructured cell niches, Adv. Sci. Weinh. Baden-Wurtt. Ger. 10 (15) (2023) e2204741, https://doi.org/10.1002/advs.202204741.

[57] A. Martinez Arias, et al., Criteria for the standardization of stem-cell-based embryo models, Nat. Cell Biol. 26 (10) (2024) 1625–1628, https://doi.org/10.1038/s41556-024-01492-x.

[58] A. Deglincerti, et al., Self-organization of human embryonic stem cells on micropatterns, Nat. Protoc. 11 (11) (2016) 2223–2232, https://doi.org/10.1038/nprot.2016.131.

[59] C.W. Gantner, B.A.T. Weatherbee, Y. Wang, M. Zernicka-Goetz, Assembly of a stem cell-derived human postimplantation embryo model, Nat. Protoc. 20 (1) (2025) 67–91, https://doi.org/10.1038/s41596-024-01042-7.

[60] N.C. Rivron, et al., Blastocyst-like structures generated solely from stem cells, Nature 557 (7703) (2018) 106–111, https://doi.org/10.1038/s41586-018-0051-0.

[61] L. Beccari, et al., Multi-axial self-organization properties of mouse embryonic stem cells into gastruloids, Nature 562 (7726) (2018) 272–276, https://doi.org/10.1038/s41586-018-0578-0.

[62] R. Li, et al., Generation of blastocyst-like structures from mouse embryonic and adult cell cultures, Cell 179 (3) (2019) 687–702.e18, https://doi.org/10.1016/j.cell.2019.09.029.

[63] S.E. Harrison, B. Sozen, N. Christodoulou, C. Kyprianou, M. Zernicka-Goetz, Assembly of embryonic and extraembryonic stem cells to mimic embryogenesis in vitro, Science 356 (6334) (2017) eaal1810, https://doi.org/10.1126/science.aal1810.

[64] S.E. Harrison, B. Sozen, M. Zernicka-Goetz, In vitro generation of mouse polarized embryo-like structures from embryonic and trophoblast stem cells, Nat. Protoc. 13 (7) (2018) 1586–1602, https://doi.org/10.1038/s41596-018-0005-x.

[65] B. Sozen, et al., Self-assembly of embryonic and two extra-embryonic stem cell types into gastrulating embryo-like structures, Nat. Cell Biol. 20 (8) (2018) 979–989, https://doi.org/10.1038/s41556-018-0147-7.

[66] B. Sozen, et al., Self-Organization of Mouse Stem Cells into an extended potential Blastoid, Dev. Cell 51 (6) (2019) 698–712.e8, https://doi.org/10.1016/j.devcel.2019.11.014.

[67] G. Amadei, et al., Inducible stem-cell-derived embryos capture mouse morphogenetic events in vitro, Dev. Cell 56 (3) (2021) 366–382.e9, https://doi.org/10.1016/j.devcel.2020.12.004.

[68] G. Amadei, et al., Embryo model completes gastrulation to neurulation and organogenesis, Nature 610 (7930) (2022) 143–153, https://doi.org/10.1038/s41586-022-05246-3.

[69] K.Y.C. Lau, et al., Mouse embryo model derived exclusively from embryonic stem cells undergoes neurulation and heart development, Cell Stem Cell 29 (10) (2022) 1445–1458.e8, https://doi.org/10.1016/j.stem.2022.08.013.

[70] K.Y.C. Lau, G. Amadei, M. Zernicka-Goetz, Assembly of complete mouse embryo models from embryonic and induced stem cell types in vitro, Nat. Protoc. 18 (12) (2023) 3662–3689, https://doi.org/10.1038/s41596-023-00891-y.

[71] S. Tarazi, et al., Post-gastrulation synthetic embryos generated ex utero from mouse naive ESCs, Cell 185 (18) (2022) 3290–3306.e25, https://doi.org/10.1016/j.cell.2022.07.028.

[72] Y. Nakaya, E.W. Sukowati, Y. Wu, G. Sheng, RhoA and microtubule dynamics control cell–basement membrane interaction in EMT during gastrulation, Nat. Cell Biol. 10 (7) (2008) 765–775, https://doi.org/10.1038/ncb1739.

[73] C. Kyprianou, et al., Basement membrane remodelling regulates mouse embryogenesis, Nature 582 (7811) (2020) 253–258, https://doi.org/10.1038/s41586-020-2264-2.

[74] M.A. Ortiz-Salazar, E. Camacho-Aguilar, A. Warmflash, Endogenous Nodal switches Wnt interpretation from posteriorization to germ layer differentiation in geometrically constrained human pluripotent cells, bioRxiv 14 (2024), https://doi.org/10.1101/2024.03.13.584912.

[75] Y.-F. Chen, et al., Control of matrix stiffness promotes endodermal lineage specification by regulating SMAD2/3 via lncRNA LINC00458, Sci. Adv. 6 (6) (2020) eaay0264, https://doi.org/10.1126/sciadv.aay0264.

[76] A. Guo, et al., Consistent apparent young's modulus of human embryonic stem cells and derived cell types stabilized by substrate stiffness regulation promotes lineage specificity maintenance, Cell Regen. 9 (1) (2020) 15, https://doi.org/10.1186/s13619-020-00054-4.

[77] J. Llewellyn, A. Charrier, R. Cuciniello, E. Helfer, R. Dono, Substrate stiffness alters layer architecture and biophysics of human induced pluripotent stem cells to modulate their differentiation potential, iScience 27 (8) (2024) 110557, https://doi.org/10.1016/j.isci.2024.110557.

[78] G.M. Anand, et al., Controlling organoid symmetry breaking uncovers an excitable system underlying human axial elongation, Cell 186 (3) (2023) 497–512.e23, https://doi.org/10.1016/j.cell.2022.12.043.

[79] C. Zhao, et al., A comprehensive human embryo reference tool using single-cell RNA-sequencing data, Nat. Methods 22 (1) (2025) 193–206, https://doi.org/10.1038/s41592-024-02493-2.

[80] P. Samal, C. Van Blitterswijk, R. Truckenmüller, S. Giselbrecht, Grow with the flow: when morphogenesis meets microfluidics, Adv. Mater. 31 (17) (2019) 1805764, https://doi.org/10.1002/adma.201805764.

[81] Y. Zheng, Y. Shao, J. Fu, A microfluidics-based stem cell model of early post-implantation human development, Nat. Protoc. 16 (1) (2021) 309–326, https://doi.org/10.1038/s41596-020-00417-w.

[82] A. Aguilera-Castrejon, et al., Ex utero mouse embryogenesis from pre-gastrulation to late organogenesis, Nature 593 (7857) (2021) 119–124, https://doi.org/10.1038/s41586-021-03416-3.

[83] E. Berger, et al., Millifluidic culture improves human midbrain organoid vitality and differentiation, Lab Chip 18 (20) (2018) 3172–3183, https://doi.org/10.1039/C8LC00206A.

[84] R.L. Brinster, Carbon dioxide production from glucose by the preimplantation mouse embryo, Exp. Cell Res. 47 (1–2) (1967) 271–277, https://doi.org/10.1016/0014-4827(67)90230-3.

[85] A.S. Lopes, et al., Respiration rates of individual bovine in vitro-produced embryos measured with a novel, non-invasive and highly sensitive microsensor system, Reproduction 130 (5) (2005) 669–679, https://doi.org/10.1530/rep.1.00703.

[86] D.B. Kolesky, R.L. Truby, A.S. Gladman, T.A. Busbee, K.A. Homan, J.A. Lewis, 3D bioprinting of vascularized, heterogeneous cell-laden tissue constructs, Adv. Mater. 26 (19) (2014) 3124–3130, https://doi.org/10.1002/adma.201305506.
[87] J.S. Miller, et al., Rapid casting of patterned vascular networks for perfusable engineered three-dimensional tissues, Nat. Mater. 11 (9) (2012) 9, https://doi.org/10.1038/nmat3357.
[88] M.A. Skylar-Scott, et al., Biomanufacturing of organ-specific tissues with high cellular density and embedded vascular channels, Sci. Adv. 5 (9) (2019) eaaw2459, https://doi.org/10.1126/sciadv.aaw2459.
[89] N. Gjorevski, et al., Tissue geometry drives deterministic organoid patterning, Science 375 (6576) (2022) eaaw9021, https://doi.org/10.1126/science.aaw9021.
[90] S. Grebenyuk, et al., Large-scale perfused tissues via synthetic 3D soft microfluidics, Nat. Commun. 14 (1) (2023) 193, https://doi.org/10.1038/s41467-022-35619-1.
[91] B. Grigoryan, et al., Multivascular networks and functional intravascular topologies within biocompatible hydrogels, Science 364 (6439) (2019) 458–464, https://doi.org/10.1126/science.aav9750.
[92] B. Cakir, et al., Engineering of human brain organoids with a functional vascular-like system, Nat. Methods 16 (11) (2019) 1169–1175, https://doi.org/10.1038/s41592-019-0586-5.
[93] K.A. Homan, et al., Flow-enhanced vascularization and maturation of kidney organoids in vitro, Nat. Methods 16 (3) (2019) 255–262, https://doi.org/10.1038/s41592-019-0325-y.
[94] J.D. Glover, et al., The developmental basis of fingerprint pattern formation and variation, Cell 186 (5) (2023) 940–956.e20, https://doi.org/10.1016/j.cell.2023.01.015.
[95] P.-F. Lenne, et al., Roadmap for the multiscale coupling of biochemical and mechanical signals during development, Phys. Biol. 18 (4) (2021) 041501, https://doi.org/10.1088/1478-3975/abd0db.
[96] N.A. Repina, et al., Optogenetic control of Wnt signaling models cell-intrinsic embryogenic patterning using 2D human pluripotent stem cell culture, Development 150 (14) (2023) dev201386, https://doi.org/10.1242/dev.201386.
[97] Z. Wang, et al., Spatial cell fate manipulation of human pluripotent stem cells by controlling the microenvironment using photocurable hydrogel, Development 151 (6) (2024) dev201621, https://doi.org/10.1242/dev.201621.
[98] P.L.H. Newman, et al., 3D printed materials with Nanovoxelated elastic moduli, Adv. Mater. 37 (15) (2025) 2416262, https://doi.org/10.1002/adma.202416262.
[99] M.J. Bissell, H.G. Hall, G. Parry, How does the extracellular matrix direct gene expression? J. Theor. Biol. 99 (1) (1982) 31–68, https://doi.org/10.1016/0022-5193(82)90388-5.
[100] S. Nemec, K.A. Kilian, Materials control of the epigenetics underlying cell plasticity, Nat. Rev. Mater. 6 (1) (2021) 69–83, https://doi.org/10.1038/s41578-020-00238-z.
[101] N. Govindasamy, H. Long, A. Ranga, B. Trappmann, I. Bedzhov, 3D biomimetic environment enabling ex utero trophoblast invasion and co-culture of embryos and somatic cells, STAR Protoc. 4 (3) (2023) 102456, https://doi.org/10.1016/j.xpro.2023.102456.
[102] S. Shibata, et al., Modeling embryo-endometrial interface recapitulating human embryo implantation, Sci. Adv. 10 (8) (2024) eadi4819, https://doi.org/10.1126/sciadv.adi4819.
[103] D. Paull, et al., Automated, high-throughput derivation, characterization and differentiation of induced pluripotent stem cells, Nat. Methods 12 (9) (2015) 885–892, https://doi.org/10.1038/nmeth.3507.
[104] X. Wang, I. Rivière, Clinical manufacturing of CAR T cells: foundation of a promising therapy, Mol. Ther. - Oncolytics 3 (2016) 16015, https://doi.org/10.1038/mto.2016.15.
[105] J. Dias, J. Garcia, G. Agliardi, C. Roddie, CAR-T cell manufacturing landscape—lessons from the past decade and considerations for early clinical development, Mol. Ther. - Methods Clin. Dev. 32 (2) (2024) 101250, https://doi.org/10.1016/j.omtm.2024.101250.
[106] L. Schiff, et al., Integrating deep learning and unbiased automated high-content screening to identify complex disease signatures in human fibroblasts, Nat. Commun. 13 (1) (2022) 1590, https://doi.org/10.1038/s41467-022-28423-4.
[107] C. Ounkomol, S. Seshamani, M.M. Maleckar, F. Collman, G.R. Johnson, Label-free prediction of three-dimensional fluorescence images from transmitted-light microscopy, Nat. Methods 15 (11) (2018) 917–920, https://doi.org/10.1038/s41592-018-0111-2.
[108] M.P. Viana, et al., Integrated intracellular organization and its variations in human iPS cells, Nature 613 (7943) (2023) 345–354, https://doi.org/10.1038/s41586-022-05563-7.
[109] N.C. Rivron, A. Martinez Arias, M.F. Pera, N. Moris, H.I. M'hamdi, An ethical framework for human embryology with embryo models, Cell 186 (17) (2023) 3548–3557, https://doi.org/10.1016/j.cell.2023.07.028.
[110] "Human stem cell-based embryo models: A review of ethical and governance questions," Nuffield Council on Bioethics,. 2024.
[111] "Code of Practice for the generation and use of human stem cell-based embryo models," Cambridge Reproduction, 2024.
[112] A. De Los Angeles, et al., Human embryo research: how to move towards a 28-day limit, Nature 643 (8070) (2025) 31–34, https://doi.org/10.1038/d41586-025-02016-9.
[113] S. Grebenyuk, A. Ranga, Engineering organoid vascularization, Front. Bioeng. Biotechnol. 7 (2019), https://doi.org/10.3389/fbioe.2019.00039. Accessed: Mar. 29, 2023. [Online]. Available:.